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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **260th Meeting** *of the Biochemical Society was held at the School of Biochemistry, Tennis Court Road, Cambridge, on Saturday,* 27 *September* **1947**, *at* 11 *a.m. when the following papers were read:*

COMMUNICATIONS

Correlation between Penicillin Resistance and the Ability to Assimilate Glutamic Acid in *Staphylococcus aureus*. By E. F. Gale. (*Biochemical Laboratory, University of Cambridge*)

Gram-positive cocci assimilate glutamic acid in the free state and concentrate it in the internal environment prior to metabolism (Gale, 1947). Penicillin impairs the assimilatory process and eventually blocks the passage of glutamic acid across the cell wall (Gale & Taylor, 1946). Organisms which are resistant to penicillin require correspondingly larger amounts of penicillin to block the assimilatory process. The internal concentration of glutamic acid achieved within the cell depends upon the external concentration when this falls below a certain level; the assimilation constant can be defined as that external concentration of glutamic acid which gives rise to an internal concentration half that attained at saturation. The reciprocal of the assimilation constant can be taken as a measure of the affinity of the cell for glutamic acid.

A study of the assimilation constants of a series of organisms of differing penicillin sensitivities shows that the two factors are related in that organisms with high penicillin resistance have large assimilation constants. The correlation was proved by culturing a strain of *Staph. aureus* in increasing concentrations of penicillin and determining its assimilation constant at resistance levels of 15, 60, 200 and 2000 units/ml. As the resistance increases the affinity of the cell for glutamic acid decreases. It follows that the cell would have to change the nature of its metabolism if it were to continue to grow at very high penicillin levels. Such a change has recently been reported by Bellamy & Klimek (1947), and the metabolism of such resistant organisms will be discussed.

It appears that there is a definite relation between the assimilation of glutamic acid by the cell and its sensitivity to penicillin, although it may be that the changes demonstrated with glutamic acid may be symptomatic of a general change in the assimilatory properties of the cell.

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The Assimilation of Amino-acids by Yeast. By E. Shirley Taylor (introduced by E. F. Gale)

Further Observations on the Steroids of Pregnant Mares' Urine. By W. Klyne and J. Y. F. Paterson. (*Department of Biochemistry, University of Edinburgh*)

Compound Z previously isolated from pregnant mares' urine as its sulphate (Klyne & Marrian, 1945; Klyne, 1946) has been identified as *allo*-pregn-16-ene-3(β)-ol-20-one by comparison with authentic material (Plattner, Ruzicka, Heusser & Angliker, 1947).

Compound Y, a saturated diol $C_{21}H_{36}O_2$, which was obtained as its sulphate (Klyne, 1946) and which is probably identical with uranediol (Marker, Rohrmann & Wittle, 1938), has been further studied. Its chromic acid oxidation product 'Y ketone', $C_{21}H_{32}O_2$, ($[\alpha]_D^{20°} = -21° \pm 1°$, chloroform) is not changed by boiling with ethanolic HCl, and on Clemmensen reduction yields a hydrocarbon (m.p. 122–124°, corr.) (cf. urane, m.p. 128°, Marker, Kamm, Oakwood, Wittle & Lawson, 1938).

The conjugates from mares' pregnancy urine which could not be purified as such were hydrolyzed with acid and the hydrolysate fractionated by the usual alkali, Girard and digitonin separations. Part of the non-phenolic fraction was insoluble in benzene.

This material after acetylation and chromatography (Al_2O_3) was fractionally crystallized from methanol and aqueous methanol (Marker, Kamm, Crooks, Oakwood, Wittle & Lawson, 1938) to yield the pregnanetriol triacetate of Haslewood, Marrian & Smith (1934), (m.p. 164–166°, corr., $[\alpha]_D^{23°} = -45° \pm 1°$, chloroform) and another compound, T acetate, which is probably the uranetriol triacetate of Marker, Kamm, Crooks *et al.* (1938):

	m.p.		m.p.
Cpd. T	296–298° (uncorr.)	Uranetriol	295–300°
Cpd. T acetate	135–136° (corr.)	Uranetriol acetate	136°
Cpd. T ketone (CrO_3 oxidation product)	223–226° (corr.)	Uranetrione	245°

Chromatography (Al_2O_3) of the non-phenolic, non-ketonic digitonin-precipitable fraction has yielded two new compounds. Compound W appears to be a saturated diol; its acetate has the probable formula $C_{25}H_{40}O_4$, m.p. 175·5–178° (corr.), $[\alpha]_D^{19°} = -54° \pm 1°$ (chloroform). These properties do not agree with those of the acetate of any known C_{21} diol having a 3(β)-hydroxy group. Compound V appears to be a saturated monohydroxy steroid, m.p. 61–63° (corr.).

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Plattner, Pl. A., Ruzicka, L., Heusser, H. & Angliker, E. (1947). *Helv. chim. Acta*, **30**, 385.

The Isolation from the Urine of Pregnant Mares of *allo*-Pregnan-3(β)-ol-20-one sulphate. By J. Y. F. Paterson and W. Klyne. (*Department of Biochemistry, University of Edinburgh*)

The urine was extracted by the method previously described (Schachter & Marrian, 1938; Klyne & Marrian, 1945). The 'water-insoluble', 98 % acetone-soluble, material gave, with piperazine dihydrochloride, the crystalline salt of a sulphate, m.p. 240–241° (dec., corr.), probable formula

$$(C_{21}H_{33}O.SO_4^-)_2.(C_4H_{12}N_2^{++}).$$

This sulphate was hydrolyzed with boiling dilute hydrochloric acid, and the product treated with digitonin. The digitonin-precipitable material, after acetylation, was chromatographed (Al_2O_3), yielding X acetate, $C_{23}H_{36}O_3$, m.p. 140–141° (corr.), $[\alpha]_D^{22°} = +72° \pm 1°$ (chloroform). X acetate was saturated to tetranitromethane, and gave a positive Zimmerman ketosteroid reaction. Hydrolysis of the acetate gave a substance X, $C_{21}H_{34}O_2$, m.p. 190–191° (corr.), $[\alpha]_D^{20°} = +90° \pm 1°$ (chloroform).

These melting-points and rotations agree with those of *allo*-pregnan-3(β)-ol-20-one and its acetate (Meystre & Miescher, 1946), which were prepared from the acetate of pregn-5-ene-3(β)-ol-20-one (Plattner, Heusser & Angliker, 1946). Compound X and its acetate on mixing with *allo*-pregnan-3(β)-ol-20-one and its acetate respectively gave no depression of melting-point.

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The Production of Propionic Acid by Decarboxylation of Succinic Acid in a Bacterial Fermentation. By A. T. Johns (introduced by M. Stephenson). (*Department of Biochemistry and the A.R.C. Unit of Animal Physiology, University of Cambridge*)

In the course of a survey of the organisms responsible for the production of propionic acid in the rumen of the sheep a new micrococcus has been isolated in pure culture. This organism is a strict anaerobe and differs from the *Propionibacteria* in that it ferments no sugar tested, but produces propionic acid along with acetic acid, H_2 and CO_2 from lactic acid. A survey of possible fermentation substrates

showed that it attacks pyruvic, oxalacetic, *l*-malic and fumaric acids with the production of propionic, acetic and carbonic acids and H_2, but that the fermentation of succinic acid gives propionic acid and CO_2 as sole products.

Barker & Lipmann (1944) have put forward evidence that lactate does not form an intermediate in the reduction of pyruvic to propionic acid. The appearance of C^{13} in the carboxyl group of propionic acid formed during fermentation by *Propionibacteria* in the presence of $C^{13}O_2$ led Wood, Werkman, Hemingway & Nier (1941) to suggest that propionic acid may arise via the Wood-Werkman CO_2 fixation scheme by the decarboxylation of one of the dicarboxylic acids, possibly succinic. Such a reaction has, however, never been shown as a result of fermentation by true *Propionibacteria*. The findings noted above with this micrococcus would be in general agreement with the scheme postulated above, and consequently attention has been concentrated on the decarboxylation of succinic acid by this organism.

Washed suspensions of cells produce a simple decarboxylation of succinic acid giving 97 % of the theoretical yield of CO_2 according to the equation

$$HOOC—CH_2—CH_2—COOH \rightarrow CH_3—CH_2—COOH + CO_2.$$

Investigation of the nature of the acid product by the chromatographic method (Elsden, 1946) gives 100·1 % yield propionic acid and no other product. The reaction can be studied manometrically at an optimum pH of 7·4 when Q_{CO_2} values of the order 40–50 are obtained at 37°.

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The Decarboxylation of β-(2:5-dihydroxyphenyl)-alanine. By H. Blaschko and G. H. Sloane Stanley. (*Department of Pharmacology, University of Oxford*)

Extracts containing the enzyme dopa decarboxylase (rat's liver and guinea-pig's kidney) will decarboxylate DL-2:5-dihydroxyphenylalanine. The rate of the reaction is slightly slower than with the 3:4-acid; the total CO_2 formation suggests that only one stereoisomeride is decarboxylated.

This observation is of interest in connexion with tyrosine breakdown. Neuberger, Rimington & Wilson (1947) have shown that in human alcaptonuria the 2:5-acid is converted to homogentisic acid; their quantitative data suggest that the L-form of the acid is an intermediate in homogentisic acid formation. The possibility of a pathway, tyrosine→2:5-dihydroxyphenylalanine→2:5-dihydroxyphenylethylamine→homogentisic acid, has therefore to be considered (see Neubauer, 1928); our results show that a mammalian enzyme exists which will catalyse one of these steps.

The acid is not markedly oxidized by the ophio-oxidase of cobra venom.

The authors are grateful to Dr A. Neuberger for the 2:5-dihydroxyphenylalanine used in these experiments.

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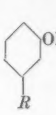
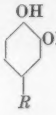
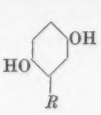
Neuberger, A., Rimington, C. & Wilson, J. M. G. (1947). *Biochem. J.* **41**, 438.

Decarboxylation of Tyrosine and its Derivatives by Mammalian and Bacterial Enzymes. By H. Blaschko and G. H. Sloane Stanley. (*Department of Pharmacology, University of Oxford*)

Tyrosine is not decarboxylated by the mammalian dopa decarboxylase, but Epps (1944) has shown that the tyrosine decarboxylase from *Streptococcus faecalis* will also act on 3:4-dihydroxyphenylalanine. An analysis of this difference shows that the mammalian enzyme requires the presence of a phenolic hydroxyl group in the *meta* position, the bacterial enzyme one in the *para* position. Both *m*-tyrosine and 2:5-dihydroxyphenylalanine are decarboxylated by extracts containing dopa decarboxylase, but not by the bacterial enzyme.

meta-Tyrosine is a substrate of ophio-oxidase of cobra venom and of D-amino-acid oxidase of pig's kidney.

The authors are grateful to Prof. I. C. Gunsalus for the strain of *Str. faecalis* (A.T.C.C. no. 4083) and to Dr H. R. Ing for the *meta*-tyrosine used in these experiments.

Substrate:	Tyrosine	*meta*-Tyrosine	3:4-Dihydroxy-phenylalanine	2:5-Dihydroxy-phenylalanine
				
Guinea-pig's kidney	−	+	+	+
Streptococcus faecalis	+	−	+	−

$R = —CH_2.CH(NH_2)COOH.$

REFERENCE

Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.

Increased β-Glucuronidase in Response to Tissue Injury. By G. A. Levvy, Lynda M. H. Kerr and J. G. Campbell (introduced by G. F. Marrian). (*Department of Biochemistry, University of Edinburgh and Royal (Dick) Veterinary College, Edinburgh*)

Fishman (1940) found that repeated feeding of menthol to mice caused statistically significant increases in β-glucuronidase in liver, spleen and kidney. He postulated this enzyme to be responsible for glucuronide synthesis *in vivo*, and suggested that he was measuring adaptation by the enzyme in response to the presence of excess substrate. Menthol is known to be excreted as the glucuronide in certain species, but it has not been established that β-glucuronidase acts synthetically.

Using a new method for assay of the enzyme (Kerr, Graham & Levvy, 1947), an attempt was made to confirm Fishman's findings. Twenty-four hours after intraperitoneal injection of *l*-menthol in oil into mice, there was a marked rise in glucuronidase in liver, but not in kidney and spleen. Gross liver damage was noted, and it was considered that this might explain the rise in the enzyme. On histological examination, fatty degeneration, necrosis and evidence of repair were seen in the liver, while the kidneys and spleen showed little deviation from normal.

A variety of substances known to produce liver or kidney damage, but in most cases unlikely to form glucuronides, were injected into mice and liver, kidney and spleen were examined histologically and for their glucuronidase content. Carbon tetrachloride and *l*-menthol-β-D-glucuronide resembled menthol in their effects. Chloroform behaved in the same way as menthol with regard to liver and spleen, but produced severe damage in kidney unaccompanied by any rise in the enzyme. Mercuric nitrate produced profound damage in kidney, followed by rapid repair and a rise in glucuronidase. It had little effect in either respect on liver and spleen. Phenylarsenoxide and yellow phosphorus caused severe liver damage with little evidence of repair. The former had no effect on the glucuronidase level in liver, and the latter depressed it, favouring the view that a rise in the enzyme is associated with repair rather than damage. The results obtained for kidney after injecting chloroform could be explained likewise.

A further series of experiments was carried out with relatively non-toxic compounds known or considered to give rise to glucuronides *in vivo*. Neither pregnanediol, sulphathiazole nor pregnanediol-β-D-glucuronide affected the glucuronidase level in liver, spleen or kidney. No pathological changes in these organs were observed after injection of the first two compounds.

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Observations on the Decarboxylation of Glutamine by *Clostridium welchii*. By H. A. KREBS

Clostridium welchii, strain S.R. 12, specifically decarboxylates glutamic acid (Gale, 1945) and glutamine (Gale, 1947). Glutamine reacts much more slowly than glutamic acid, especially at low concentrations of the substrate. At pH 4·5, for example, glutamic acid reacted quantitatively in 15 min., whilst glutamine required 80 min. At pH 4·0 the differences were even greater. The rate of reaction of glutamine is much accelerated by the detergent cetyltrimethyl ammonium bromide ('cetavlon'), 0·02%. At pH 4·0 the acceleration was about 20-fold. Plasma protein, or protein containing tissue extracts, also accelerated the decarboxylation of glutamine, though much less than cetavlon. The decarboxylation of glutamic acid was also accelerated by cetavlon, but owing to the relatively high rate of the decarboxylation without detergent the percentage increase was in this case smaller than with glutamine. An anionic detergent, sodium dodecylsulphate, inhibited the decarboxylation of glutamine and glutamic acid. In another strain of *Cl. welchii* which contained histidine and glutamic decarboxylases cetavlon likewise accelerated the glutamic decarboxylase but inhibited the histidine decarboxylase, and sodium dodecylsulphate had no effect on the histidine decarboxylase.

The decarboxylation of glutamine is preceded by the conversion into ammonium glutamate. The glutaminase of strain S.R. 12 is highly specific. No other substance has so far been found to yield ammonia when incubated with a washed suspension of the organism at pH 4·9. This glutaminase preparation is more convenient to make than Archibald's (1944) glutaminase preparation from kidney and is more specific. It can be used for the determination of glutamine on Archibald's principle.

An acceleration of the decarboxylation of glutamic acid and glutamine by cetavlon was also found in *Escherichia coli* and *Proteus morganii*.

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Paper Chromatography of Flavin Nucleotides. By J. L. CRAMMER

Adenosinetriphosphate in Glutamine Synthesis. By W. H. ELLIOTT (introduced by M. DIXON).
(*Biochemical Laboratory, University of Cambridge*)

It was shown by Krebs (1935) that the energy required for the synthesis of glutamine from glutamic acid and NH_3, which occurs in tissue slices of brain cortex, can be furnished by the oxidation of glucose. During the course of a recent survey for phosphate transferring enzymes, evidence has been obtained that adenosinetriphosphate (ATP) is the immediate energy donor for glutamine synthesis. Since this work was performed, Speck (1947), in a recent publication, has reported similar results obtained with pigeon liver.

An enzyme which synthesizes glutamine has been extracted from acetone-dried grey matter of sheep's brain. The system requires the presence of Na (L)-glutamate, NH_3 and ATP. When hydroxylamine is added instead of NH_3, the corresponding hydroxamic acid is formed, which can be conveniently estimated colorimetrically by the method of Lipmann & Tuttle (1945). Using an enzyme preparation freed from adenosinetriphosphatase activity, it was shown that the formation of hydroxamic acid is accompanied by the liberation of an equivalent amount of inorganic phosphate from ATP. A similar liberation of phosphate occurs in the presence of NH_3 during the formation of glutamine.

It seems possible that the first stage of glutamine synthesis might be a transfer of phosphate from ATP to the γ-COOH of glutamic acid forming glutamyl-phosphate. This could then react with either NH_3 or hydroxylamine to form glutamine or hydroxamic acid respectively. The enzyme resembles known phosphokinases in requiring magnesium ions and cysteine for its activity. It is completely specific for the natural isomer of glutamic acid.

The enzyme has also been found in a cell-free extract obtained from *Staphylococcus aureus*. The bacterial enzyme preparation is completely inhibited by crystal violet in a concentration of 1 in 10,000 (0·00025M). This can be correlated with the results obtained by Gale & Mitchell (1947), who showed that crystal violet and other dyes of the triphenylmethane series suppress some phase of glutamic acid metabolism. Methionine-sulphoxide also inhibits the enzyme.

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Speck, J. F. (1947). *J. biol. Chem.* **168**, 403.

The Amino-acid Composition of Salmine. By G. R. Tristram. (*School of Biochemistry, University of Cambridge*)

REFERENCE

Tristram, G. R. (1947). *Nature, Lond.*, **160**, 637.

Persistence of Suramin Analogues in the Blood Stream. By A. Spinks. (*I.C.I. Ltd., Blackley, Manchester*)

In the hope of correlating the remarkable persistence of suramin with its molecular structure, eleven related compounds have been studied. They include the three constituent amino-acids of suramin, and peptide-like compounds and ureas of varying complexity prepared from these. Methods of determining each have been devised, and each has been administered to rabbits intravenously in doses of 100 mg./kg. The resulting blood concentration-time curves show two main trends. First, persistence tends to increase with increasing molecular complexity; and, secondly, compounds containing naphthylaminepolysulphonic acids as end-groups tend to show higher persistence than analogous carboxylic acids. For persistence of the suramin order the presence in the molecule of two naphthylaminepolysulphonic acid groups, and a total number of six amino-acid residues appears to be necessary. *m*-Aminobenzoic, *m*-amino-*p*-toluic, and sulphanilic acid groups may be interchanged within this basic design without marked effect on the persistence.

Since the compounds examined include most of the possible hydrolysis products of suramin, and since these were rapidly eliminated it is probable that suramin is not markedly hydrolyzed *in vivo*. No relation between persistence and activity was found. It is probable that these two properties are conferred by different structural features of the molecule.

The Estimation of Suramin (Antrypol, Bayer 205) in Plasma. By J. C. Gage, F. L. Rose and Mary M. Scott. (*I.C.I. Ltd., Blackley, Manchester*)

A colorimetric method of estimating suramin has been developed, based on an observation that the red colour of certain styryl-quinoline dyes is discharged by the drug. Plasma proteins do not interfere with the reaction; the estimation of suramin in plasma is simpler and more rapid than the hydrolysis method of Dangerfield, Gaunt & Wormall (1938) and attains a higher sensitivity. The plasma concentration curve obtained by this method from a rabbit dosed intravenously with the drug is similar to that obtained by the hydrolysis method. A number of the possible hydrolytic fission products of suramin have been examined and positive results by the method have been found with those containing the naphthalene trisulphonic acid residue.

REFERENCE

Dangerfield, Gaunt & Wormall (1938). *Biochem. J.* **32**, 59.

Changes in Nicotinic Acid Content of Cereals occurring during Germination. By CHLOE KLATZKIN, F. W. NORRIS and F. WOKES. (*Ovaltine Research Laboratories and the Department of Industrial Fermentation, University of Birmingham*)

James, Norris & Wokes (1947) have recently described a chemical method of estimating nicotinic acid which gives fairly good agreement with microbiological assays on a variety of foods. This chemical method has now been used to estimate nicotinic acid in different cereals before and after germination, in parallel with microbiological assays.

Barley, wheat, maize and oats have been malted by the large-scale procedure normally used for barley in this country, and the nicotinic acid content determined by chemical and microbiological methods at intervals during the process of malting. All these cereals, and rice, have also been germinated in the laboratory, at room temperature and at 37°, and their nicotinic acid content estimated. Changes in the average dry weight per seed have been determined, and correction for these changes applied when calculating the nicotinic acid content per seed.

Using both chemical and microbiological assays, no increases in the nicotinic acid content have been observed during the germination of barley or maize and only slight increases during the malting of wheat. This agrees with the findings of Davis, Laufer & Saletan (1943), who used microbiological methods only. Appreciable increases have been found to occur during the germination of oats and rice.

The very large increases found to occur in oats by American workers (Burkholder, 1943; McVeigh, 1944), using microbiological methods of assay only, have not been confirmed. These large increases may be due to their having obtained a more rapid growth of seedlings, since the average loss in dry weight per seed during 5 days in their experiments is much greater than the corresponding loss during 12 days in our experiments.

However, experiments on oats, barley and maize germinated at 37° in our laboratory failed to show any greater increase in nicotinic acid content per seed than we had obtained under normal malting conditions. This was in spite of our obtaining a greater degree of germination, as shown by weight changes and increases in length of the shoot (acrospire).

Emphasis is laid on the importance of noting the degree of germination, by measuring acrospire ratios (A.O.A.C. 1945). With rice in particular, seeds which had germinated further during 9 days had a considerably higher nicotinic acid content per seed than those which had germinated less in the same period.

A marked discrepancy has been found between chemical and microbiological results when assaying nicotinic acid in oats. Further work is being carried out on this problem. It is concluded that some precursor of nicotinic acid is present in wheat, rice and oats.

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The Fate of 2:4-Dimethyl-3-carbethoxy-pyrrole-5-carboxylic Acid and 2:4-Dimethyl-5-carbethoxy-pyrrole-3-carboxylic Acid in the Rabbit. By C. H. GRAY and E. F. J. THORPE. (*Biochemical Department, King's College Hospital, S.E.* 5)

2:4-Dimethyl-3-carbethoxy-pyrrole-5-carboxylic acid in the form of its sodium salt was dissolved in water and administered by stomach tube to adult male rabbits fed on a stock diet. The urine subsequently collected was found to give a positive reaction for pyrroles and to reduce Benedict's solution. A glucuronide was isolated by extraction of the urine with methyl ethyl ketone after saturating with sodium chloride and acidification with hydrochloric acid. The ketone layer was washed with a little water and taken to small volume *in vacuo* when fractional precipitation with ether gave a reasonably pure specimen of the glucuronide. Recrystallization from hot water yielded the glucuronide m.p. 163°, equivalent weight 382 (theoretical 387). Diazomethane gave a methyl ester m.p. 171°. The glucuronide was soluble in the lower aliphatic alcohols and acetone but insoluble in most other organic solvents with the exception of hot ether, in which it was slightly soluble and from which it could be crystallized in the form of needles. Benedict's solution was readily reduced. The

glucuronide became rapidly discoloured on contact with the atmosphere, particularly in aqueous solution.

The isomeric 2:4-dimethyl-5-carbethoxy-pyrrole-3-carboxylic acid was likewise fed to rabbits, and it was found possible by a similar technique to isolate the corresponding glucuronide. The 3-glucuronide (m.p. 146°, methyl ester 184°) was more soluble in water and not quite as stable as the 5-isomer.

Molecule for molecule the colour produced by the glucuronide with Ehrlich's *p*-dimethyl-aminobenzaldehyde reagent was equivalent to that produced by the original acid. The total pyrroles present in the urine were therefore determined using the colour developed with Ehrlich's reagent, and the increase in excretion of glucuronic acid also measured by the method of Hanson, Mills & Williams (1944).

About 50% of the dose fed was excreted as the glucuronide in the case of 2:4-dimethyl-3-carbethoxy-pyrrole-5-carboxylic acid and about30 % in the case of 2:4-dimethyl-5-carbethoxy-pyrrole-3-carboxylic acid; in the latter case about 20% of the dose could be recovered unchanged. The fate of the remainder of the administered material is at present unknown, but it is probably metabolized.

Exp.	Dose (g.)	Amount of glucuronide recovered: Using Ehrlich's reagent (g.)	Amount of glucuronide recovered: By naphtho-resorcinol method (g.)
2:4-Dimethyl-3-carbethoxy-pyrrole-5-carboxylic acid			
1	1·6	1·5	1·2
2	1·0	0·84	0·97
3	1·3	0·55	0·7
2:4-Dimethyl-5-carbethoxy-pyrrole-3-carboxylic acid			
1	1·6	0·98	0·72
2	1·6	0·8	1·14
3	1·6	1·08	1·28

Only traces of pyrrolic material could be detected in the faeces. No urobilinoid or bilirubinoid substances could be detected in the urine using Schlesinger's and Fouchet's tests.

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The Preparation of Coproporphyrin III from Toxic Filtrates of *Corynebacterium diphtheriae* Cultures. By C. H. Gray and L. B. Holt. (*Biochemical Department, King's College Hospital, S.E. 5 and the Wright-Fleming Institute for Microbiology, W. 2*)

Corynebacterium diphtheriae was grown on a slightly modified Mueller's hydrolyzed casein medium, and after 7 days' growth the organisms were filtered off. The toxic supernatant fluid was treated with a magnesium hydroxide gel, and after standing the resulting precipitate, which contained all the porphyrin, was separated. The supernatant possessed an almost undiminished *Lf* value and was subsequently used for the preparation of toxoid by treatment with formaldehyde. The red magnesium hydroxide precipitate was then treated with 10N-hydrochloric acid, and the porphyrin solution thus obtained was separated from the protein precipitate by centrifuging. A second hydrochloric acid extract of the protein precipitate was added to the main porphyrin solution, which was then adjusted to pH 3·3 by the addition of sodium hydroxide solution. The porphyrin precipitated quantitatively and was centrifuged off. After two repetitions of this precipitation at pH 3·3 from hydrochloric acid solution, the porphyrin was centrifuged off, dried *in vacuo* and esterified with methyl alcohol saturated with hydrochloric acid gas. After transferring the ester to chloroform in the usual way, the chloroform solution was washed with water, dilute ammonia and again with water and then evaporated to dryness to give the crude ester.

The crude ester was dissolved in benzene and the solution passed through a column of calcium carbonate. The bulk of the porphyrin ester passed slowly through the column as an entity and proved to be pure coproporphyrin III tetramethyl ester with the properties previously described (Gray & Holt, 1947). A second zone left on the column required for elution benzene containing 20% (v/v) chloroform, and proved to be uroporphyrin I octamethyl ester, m.p. 288°. A third zone required pure chloroform for its elution, and although possessing the properties of a porphyrin ester could not be identified. A fourth brown zone left on the column was not a porphyrin.

From 130 l. of *C. diphtheriae* filtrate, 479 mg. of pure coproporphyrin III ester and 7 mg. of pure uroporphyrin I ester were isolated. Since these materials are readily obtainable without significant loss of toxin, the preparation of coproporphyrin III

ester in large quantities in the large-scale production of diphtheria toxoid provides a new and hitherto unexplored source of coproporphyrin III. In the large-scale preparation of purified diphtheria toxoid, it is more convenient to perform the magnesium hydroxide precipitation after conversion of the toxin to toxoid.

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The Formation of Nicotinamide by *B. coli*. By P. Ellinger and M. M. Abdel Kader. (*Lister Institute, London, S.W.* 1)

The synthesis of nicotinamide or nicotinic acid by a number of bacteria in pure culture has been shown by Burkholder & McVeigh (1942) and Thompson (1942). Recently Ellinger & Emmanuelowa (1946) have shown that intestinal biosynthesis of nicotinamide in human beings is mainly due to *B. coli*, and Ellinger, Abdel Kader & Emmanuelowa (1947) have examined *in vitro* this synthesis by *B. coli*.

To study the mechanism of this biosynthesis various strains of *B. coli* were incubated in ammonium lactate (Fildes, 1938), and the nicotinamide formed was measured microbiologically (Barton-Wright, 1944). Then one of the following amino-acids was added before incubation: L(+)-ornithine, L(+)-arginine, D, L-glutamine, D, L-glutamic acid, L(+)-citrulline, L(+)-proline, L(+)-leucine, L(−)-isoleucine, D, L-valine or D, L-tryptophane. The examination for nicotinamide formed revealed that concentrations of 10^{-3} to 6×10^{-4}M of ornithine increased the nicotinamide formation up to $4\frac{1}{2}$ times, while glutamine and arginine gave an increase of 50–70% when compared with the synthesis of nicotinamide in the control. All the other amino-acids either did not affect the nicotinamide formation or inhibited it. With the help of a strain of *Leuconostoc mesentroides*, kindly supplied by Dr B. C. Johnson of the University of Illinois, which was less sensitive to nicotinamide than nicotinic acid, we could establish that almost all the synthesized material was nicotinamide and not nicotinic acid. Preliminary experiments with washed and disintegrated *B. coli* showed that in saline solution some nicotinamide was formed from ornithine. This formation was inhibited by NaCN (10^{-4}M). The experiments confirm the suggestion of Guggenheim (1940) that ornithine might be the basis of the biosynthesis of nicotinic acid.

Whether this mechanism is involved in the formation of nicotinamide from tryptophane and whether ornithine is formed from tryptophane needs further investigation. Tryptophane did not increase the formation of nicotinamide by *B. coli*. This might be due to the fact that *B. coli* are unable to disintegrate tryptophane to ornithine. The results of preliminary experiments carried out with a mixed culture of intestinal bacteria from the rats' caecum support this hypothesis. If an emulsion of this culture is incubated in Barton-Wright medium with tryptophane an increased biosynthesis of nicotinamide is observed.

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The Biosynthesis of a Member of the Folic Acid Group of Factors. By D. A. Hall. (*School of Medicine, Leeds* 2)

Enzyme systems capable of deaminating histidine are reported by Edlbacher & Neber (1934) as present in liver, and by Borghi & Tarrantino (1938) in skin tissue. Members of the folic acid group of factors have long been known to be present in liver tissue, and the present author has also shown that skin tissue contains folic acid-active material. In view of this correlation and the observations previously reported (Hall, 1947) regarding the production of a member of the folic acid group by chemical means from histidine, it was considered desirable to determine whether certain components of the enzyme

systems mentioned above were capable of producing activity from histidine.

It was shown that an aqueous extract of mouse skin, when incubated at pH 6·74 at 37° with histidine, was capable of stimulating the growth of cultures of *S. Lactis* R at lower concentrations than when treated under similar conditions in the absence of histidine. A tenfold increase in activity was obtained after 5 hr. incubation. Similar results were obtained with aqueous or M/20-phosphate buffer (pH 6·74) extracts of both mouse and cat liver, and the effect was shown to be truly enzymatic, and not due to liberation of bound activity, by the inactivation of the preparations by boiling. Attempts to separate the responsible components from the total liver extract are not complete, but it has been shown that the activity is not connected with that fraction soluble in half-saturated ammonium sulphate solution containing vitamin B_c conjugase. This fraction is, however, required as well as the precipitate for the reaction to proceed. Edlbacher & Neber (1934) and Walker & Schmidt (1944) postulated the production of unstable intermediates of the type-formamidino glutaric acid or formyl glutamine, and it may be possible that other components of the liver extract are capable of catalyzing the re-combination of small quantities of these intermediates with the production of pterin-like nuclei. Arginine, which is of similar structure to these intermediates, will also act as a substrate for the production of folic acid like activity, but glutamic acid, the end-point of the degradation of histidine, is not equally effective.

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DEMONSTRATIONS

Some Characteristics of Penicillin-resistant Staphylococci. By E. F. Gale

A New Micro-electrophoresis Cell. By P. D. Mitchell (introduced by E. Baldwin)

The **261st Meeting** *of the Biochemical Society was held at St Thomas's Hospital Medical School, London, S.E.* 1, *on Friday,* 31 *October* 1947, *at* 2.15 *p.m., when the following papers were read:*

COMMUNICATIONS

An Apparatus for the Preparative Electrophoretic Separation of Amino-acids, Peptides and Proteins. By J. A. V. Butler and Joan M. L. Stephen. (*Courtauld Institute of Biochemistry, The Middlesex Hospital, W.* 1)

Nearly all of the considerable number of arrangements which have been proposed for separating substances having different electric mobilities have serious disadvantages of one kind or another. Diaphragm cells as used by Gordon, Martin & Synge (1941), among others, with three or more compartments, are useful for rough separations of substances moving in opposite directions, but are useless for taking advantage of differences of mobility in the same direction since convective mixing occurs in the cells and the useful separative process is confined to the membranes. The Tiselius apparatus is essentially one for the analysis of the moving fronts, and though samples can be withdrawn for analysis it does not permit the complete separation of the components of a mixture (Theorell, 1934; Svensson, 1946; Bunn, 1947). Various methods of stopping convection on the electrolyte column have been suggested, e.g.

silica gel (Consden, Gordon & Martin, 1946), which has been effectively used for separation of amino-acids and the lower peptides; the use of tubes packed with sintered glass (Coolidge, 1939), which however, confers a very high resistance on the column.

We have developed an apparatus in which migration occurs down a tube, which is packed with a loose filling material, such as asbestos fibre of the type used in Gooch crucibles, glass wool, or cotton fibres. These do not greatly increase the resistance of the column of electrolyte and effectively prevent convection. The filter can be divided into sections by filter-paper barriers at 1 in. intervals, which permit the wads to be extracted from the tube and easily separated from each other. The liquid contents of each segment can then easily be squeezed into a test-tube for analysis.

The tubes (of a polystyrene plastic material) are attached to completely closed silver chloride electrodes of the type used with the Tiselius apparatus so that there is no electro-endosmotic flow through the apparatus. Good separations have been obtained with mixtures of amino-acids, of amino-acids and peptides and of two proteins. In the latter case glass wool or cotton had to be used as the proteins were adsorbed on asbestos.

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Synthesis of Phospholipids in the Foetus. By G. Popják, Beit Memorial Fellow for Medical Research. (*Department of Pathology, St Thomas's Hospital Medical School, London, S.E.* 1)

Two to six hours after pregnant rats, rabbits and guinea-pigs were injected with Na_2HPO_4 labelled with P^{32}, the specific activity of phospholipid-P extracted from foetal livers and foetal placentae of all three species was much greater than that of the maternal plasma phospholipid-P. If foetal phospholipids were derived from the preformed maternal substances by placental transmission only, the specific activity of foetal phospholipids should not exceed the activity of the maternal plasma lipid. The findings, therefore, indicate that the foetal liver and foetal placenta synthesize phospholipids. When the mothers were injected with the labelled PO_4 the radioactivity of the phospholipids extracted from foetal tissues other than liver and placenta was less than that of the maternal plasma lipid. The lower specific activity of these foetal phospholipids was due to the fact that only very small amounts of labelled inorganic PO_4 had accumulated in these tissues. In a few experiments the foetuses were injected with labelled Na_2HPO_4 directly *in utero*. In these experiments no radioactivity could be detected in the maternal plasma phospholipids, yet all foetal tissues examined contained labelled lipids. Preliminary measurements indicate that the turn-over rate of phospholipids in the foetal liver and placenta is higher than in the maternal liver; in other foetal tissues, however, it is approximately the same as in the maternal liver, which is the most active organ in the adult animal in this respect.

Separation of Acidic Amino-acids by Means of a Synthetic Anion Exchange Resin. By R. Consden, A. H. Gordon and A. J. P. Martin. (*Wool Industries Research Association, Leeds* 6)

The synthetic anion exchange resin, Amberlite IR-4 was used by Cannan (1944) to separate aspartic and glutamic acids together from the other amino-acids in a protein hydrolyzate. By using the resin chromatographically at a suitable pH, the method has been extended to separate aspartic from glutamic acid. Where these amino-acids are present in a protein hydrolyzate, a preliminary separation from the basic and neutral amino-acids is necessary. This is achieved on a chromatogram of the resin maintained at pH 3–4, which preferentially retains the dicarboxylic amino-acids. At this low pH, the resin is sufficiently charged to compete successfully with the basic amino-acids for the acidic amino-acids. After elution with N-HCl, the dicarboxylic acids (up to 5 mg./g. resin) are transferred to a chromatogram at pH 2·5. At this pH, the glutamic acid is first eluted, followed by aspartic acid. The progress of elution in both stages is followed by partition chromatography on paper (Consden, Gordon & Martin, 1944) of small samples of the eluates. Recoveries of 91 and 96% respectively, of glutamic and aspartic acids were obtained from a synthetic mixture simulating a wool hydrolyzate, using the copper phosphate titration

method of Pope & Stevens (1939). If cysteic acid is present, this may be separated after elution of the dicarboxylic amino-acids at pH 2·5 by elution with N-HCl. Alternatively, cysteic acid, because of its much higher net charge at low pH, may be separated in one stage, employing a chromatogram at pH 1·5. In this way, and working on a sufficiently large scale, cysteic acid has been isolated from hydrolyzates of chlorinated wool and was found to be optically active.

These separations take place because of the difference in net charge between the acidic amino-acids at a given pH. In addition to the above factors, successful separations depend on (*a*) the maintenance of a uniform pH in that part of the column in which separations take place, and (*b*) the rate of flow through the columns being kept sufficiently low to ensure equilibration. This is aided by the use of finely ground resin.

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The Site of Formation of Gastric HCl as Determined by Indicators. By Norah M. Bradford and R. E. Davies. (*M.R.C. Unit for Research in Cell Metabolism, The University, Sheffield* 10)

Claude Bernard (1858) first used microchemical methods in an attempt to locate the site of formation of the HCl produced by gastric mucosa, and Babkin (1944) has reviewed the large amount of work on this topic. There is no convincing evidence for the presence of acid in fresh excised tissue and, although it is generally held that the oxyntic cells secrete HCl at concentrations isotonic with the blood (see Hollander, 1943), some workers (see Hoerr & Bensley, 1936) have stated that a precursor of the acid occurs within the gastric tubules and is hydrolyzed to form HCl in the foveolae.

Frog gastric mucosa can secrete some dyestuffs *in vivo* (Henning, 1932) and HCl *in vitro* (Delrue, 1930). Tied bags of isolated frog gastric mucosa (Davies, 1946) were used to find out which dyestuffs were secreted without interfering with respiration or acid secretion. All of the following basic (chromogen in cation) dyes, of widely differing chemical structure, were transported into the lumen of the mucosal bag from nutrient saline solutions: acridine, acriflavine, azur I, azur II, benzene azo-α-naphthylamine, Bismarck brown, brilliant cresyl blue, brilliant green, dimethyl yellow, malachite green, methylene blue, neutral red, rhodamine B, rhodamine 6G, safranine O, thionine, toluene azo-amino-toluene-2:1:1:4:3 and toluidine blue.

Some of these dyes are acid-base indicators, and microscopic observations were made of small pieces of isolated frog and polecat mucosa which were secreting dye and acid. With neutral red it was found that the lower regions of the mucosa and the cytoplasm of the oxyntic cells were slightly alkaline (pH 7·2–7·6), whilst the canaliculi and the lumen of some of the tubules and the secretory surface of the mucosa were acid (pH $< 6{\cdot}8$). The green fluorescence of acridine in ultra-violet light showed that the contents of some tubules and canaliculi had pH values of $< 4{\cdot}85$, whilst the red colour found when toluene-azo-amino-toluene-2:1:1:4:3 was used indicated a pH $< 1{\cdot}4$ (Kolthoff, 1932). In no case were all the gastric glands found to be actively secreting acid. The site of formation of the acid appeared to be at or near the wall of the canaliculi of the oxyntic cells in the isolated mucosa of both frogs and polecats.

The acid (chromogen in anion) dyes, congo red and phenolphthalein, were not transported, but bromthymol blue, cresol red, fluorescein and methyl orange were sometimes found in traces inside the mucosal bags, especially if their secretions were alkaline or only slightly acid.

Since phenolphthalein is not secreted and is virtually unionized, whilst acriflavine is secreted and is virtually completely ionized at pH 7·4 and below, the theory of Visscher (1942), that dyes pass through cell membranes in undissociated form, does not appear to hold in this case.

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Secretion of Water by Isolated Gastric Mucosa. By R. E. DAVIES and C. TERNER. (*M.R.C. Unit for Research in Cell Metabolism, The University, Sheffield* 10)

An apparatus has been made and used to study the rates of fluid secretion and of respiration of the isolated gastric mucosa of frogs and toads at 25°. It consists essentially of a constant pressure manometer (modified from Dixon, 1943) and a modified Warburg cup, provided with a hollow stopper to which a graduated capillary side-tube is attached. The manometer is designed to maintain the pressure acting on the nutrient surface of a sheet of mucosa (Davies, 1946) (tied secretory side upwards to the open end of the stopper) at any desired value and to measure the rate of respiration. The volume of liquid secreted is determined from the movements of liquid paraffin in the side-tube.

No secretion of water or acid was observed anaerobically, but small amounts of a neutral liquid traversed the mucous membrane in the direction of applied hydrostatic pressures. Under aerobic conditions both secreting and non-secreting mucosae were largely independent of applied pressures (of the order of ± 50 mm. H_2O). One mucosa produced even 18 cu.mm. secretion/mg. dry wt./hr. against a resultant pressure of 120 mm. H_2O. Neutral red, placed in the nutrient solution, was transported across the mucosa against external pressures.

The addition of thiocyanate (0·01–0·02 M) did not affect the increase of the O_2 uptake of the mucosa which followed stimulation by 5×10^{-5} M-histamine, but strongly inhibited the rate of acid production. The secretion of H_2O was also inhibited; H_2O often 'leaked' a little in the direction of the external pressure for up to 1 hr. before the mucosa resumed secretion at lower but steady rates.

Our results disprove the statement made by Davenport & Fisher (1940) that the energy required for the movement of H_2O 'is doubtless derived from the difference in hydrostatic pressure between the blood and the gastric juice', and are evidence that the transport of H_2O by gastric mucosa is an active secretory process.

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Absorption of Ammonia from the Rumen of the Sheep. By I. W. MCDONALD (introduced by E. BALDWIN). (*Biochemical Laboratory, University of Cambridge*)

Ammonia in the ruminal contents is derived from the deamination of food proteins and from the conversion of urea in the saliva; a series of analyses showed approximately 10 mg. urea-N/100 ml. saliva. Special interest attaches to the fate of urea in the rumen since urea has been exploited as a source of nitrogen in the nutrition of ruminants; this subject has been reviewed by Smith (1945).

Ammonia could disappear from the ruminal contents (*a*) by utilization by bacteria for their growth, (*b*) by passage in the ruminal ingesta from rumen to abomasum or (*c*) by absorption. Since ammonia-N absorbed from the rumen is probably valueless to the animal for its metabolism, the possibility of absorption assumes some importance.

In experiments on six sheep, it was found that venous blood draining the rumen contained on an average 1·7 mg. ammonia-N/100 ml., while peripheral blood from the jugular vein or carotid artery contained only traces of ammonia. It was further shown that removal of the contents from the rumen resulted in reduction of the ammonia content of the blood draining the organ, while addition of a solution of ammonium acetate to the emptied organ resulted in the reappearance of ammonia in the blood.

Since urea is rapidly converted into ammonia in the rumen (Lenkeit & Becker, 1938), it is now evident that a nitrogen cycle is established in the normal digestive process of the ruminant, viz. urea in saliva → ammonia in ruminal ingesta → ammonia absorbed into portal blood stream → converted into urea by the liver → urea in the general circulation → urea in saliva. Quantitatively it appears that, in the sheep, the amount of ammonia-N absorbed from the rumen will appreciably exceed that of urea-N secreted in the saliva.

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Separation and Estimation of Saturated, Steam-volatile Fatty Acids by Buffered Partition Columns. By R. SCARISBRICK, E. BALDWIN and V. MOYLE. (*Biochemical Laboratory, University of Cambridge*)

The chromatographic method introduced by Elsden (1946) permits the titrimetric estimation of butyric, propionic and acetic acids (the latter by difference) after their separation from mixtures by means of columns of silica gel impregnated with bromcresol green. A new partition method has now been devised in which heavily buffered silica columns are used. No indicator is employed on the columns, and the analysis is made by titration with N/200-KOH of serial samples of chloroform-butanol elutriates. Naturally occurring fatty acids of the saturated series from acetic to caprylic (C_8) can be separated and estimated in this way, except that no separation is possible between those isomeric forms of butyric and higher acids that have been tested. Caprylic acid is not separable from higher acids, but the bulk of the latter are eliminated by a preliminary steam distillation. Three columns, buffered to different pH's, are required to cover the useful range of the method. The new procedure has considerable advantages over its predecessor; the performance of the columns is not influenced by minor variations in the properties of different samples of silica, the range is substantially greater and the method has considerably greater flexibility. Each acid shows strongly characteristic behaviour on the columns, so much so, indeed, that behaviour may be taken as evidence of identity, except that isomeric pairs, e.g. *n*- and *iso*-valeric acids, cannot be distinguished in this way.

The optimal working load is about 0·1 mmol. of each acid, but the method is still sensitive with loads of only 0·01 mmol. or even less. Loads up to 1 mmol. can be handled, though the separations between adjacent acids are then incomplete. Under optimal conditions $100 \pm 4\%$ of the acids are recoverable, whether put on the columns singly or in the form of mixtures.

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Nucleic Acids Labelled with P^{32} and N^{15}. By J. N. DAVIDSON and W. RAYMOND. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

Sodium phosphate containing P^{32} was administered to rats in doses of about 10 μc. per animal and the livers were removed 2–4 hr. later. Cytoplasmic and nuclear material were separated and the nucleic acids isolated from each fraction. The phosphorus of the ribonucleic acid was more active than that in the deoxyribonucleic acid, the ratio of the specific activities being about 7 : 1. Similar results were obtained with rabbits. Brues, Tracy & Cohn (1944) obtained figures of this order, while even higher ratios were found by Hammarsten & Hevesy (1946).

In regenerating rat livers the activity of both types of nucleic acid was increased but especially that of the deoxyribonucleic acid, so that the ratio of the specific activities fell to between 2 and 3. In foetal liver the specific activity of the deoxyribonucleic acid frequently exceeded that of the ribonucleic acid.

When ammonium citrate containing 4·18 atom % excess N^{15} was fed to pigeons and rats in doses of 50 mg./day for 3 days, the ribonucleic acid isolated from the pigeon liver contained 0·048 atom % excess N^{15} and the deoxyribonucleic acid only 0·003 atom % excess. The figure for the uric acid excreted in the last 24 hr. was 0·204 atom % excess N^{15}. The ribonucleic acid isolated from rat liver contained 0·053 atom % excess N^{15}, while the amount of excess N^{15} in the deoxyribonucleic acid was negligible.

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The Nucleic Acid Content of Fibroblasts Growing *in vitro*. By J. N. Davidson, I. Leslie and C. Waymouth. (*Biochemistry Department, St Thomas's Hospital Medical School, London, S.E.* 1)

In fresh explants of the 12-day chick embryo heart cultivated in plasma clot in roller tubes (Davidson & Waymouth, 1943, 1945, 1946), the ribonucleic acid phosphorus (RNAP) content is 2–3 times greater than the deoxyribonucleic acid phosphorus (DNAP) content. Cultures incubated for 24–48 hr. in Tyrode solution alone lose as much as 40 % of both types of nucleic acid. The addition of a mixture of embryo extract and serum at this stage is followed by a marked rise in RNAP. Increases in DNAP occur but are less pronounced, with the result that the RNAP : DNAP ratio rises. Regular fluctuations in the RNAP level occur during the first 24 hr. after the addition of growth-promoting media.

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Bilirubin-Serum Protein Complexes. By N. H. Martin (introduced by F. T. G. Prunty). (*St George's Hospital Medical School, London, S.W.* 1)

Bennhold in 1932 demonstrated the migration of bilirubin with the albumin fraction of human serum. The ultracentrifugal studies of Pedersen & Waldenström (1937) seemed to confirm this, and it has, since then, been concluded that the 'bound bilirubin' circulating in the blood stream was attached to the albumin (Watson, 1946).

While studying the changes in electrophoretic pattern of the serum proteins in infective hepatitis (Martin, 1946) it was observed repeatedly that the bilirubin present did not migrate with the same mobility as the albumin. The relationship of bilirubin to the circulating serum proteins was therefore reinvestigated. The methods of fractionation elaborated by Cohn (1946) and his collaborators made it possible to study the behaviour of bilirubin with separate proteins and groups of proteins existing in the human blood stream.

Three approaches were employed:

(*a*) A study of the alteration in the characteristic absorption spectrum of bilirubin in the presence of the protein under test.

(*b*) A study of the migration of bilirubin in a standard electrical field in the presence of the protein under test.

(*c*) A study of the behaviour of the bilirubin in the presence of protein in a two-chambered system the chambers being separated by a semipermeable membrane impermeable to the protein.

Using these techniques it was concluded that bilirubin formed a complex with albumin in a molecular ratio of the order of 2 mol. of bilirubin to 1 mol. of albumin at a pH of 7·4 up to 3 mol. of bilirubin to 1 mol. of albumin observed at a pH of 7·9. This ratio could be altered by previous alteration of amino groups in the albumin molecule, using methyl isourea in a modification of the method of Greenstein (1925).

Interaction also appeared to occur with an α_1 globulin fraction and two α_2 globulin fractions. It did not occur to any significant extent with a purified β globulin nor with an electrophoretically homogeneous γ globulin.

It appears that the present conception must be modified and that under certain conditions in addition to albumin a significant percentage of bilirubin circulating in the blood stream may be bound to the α globulins.

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Folic Acid, B_c Conjugate, B_T and Anti-anaemia Liver Concentrates in the Nutrition of the Mealworm, *Tenebrio molitor*. By G. FRAENKEL. (*Department of Zoology and Applied Entomology, Imperial College, London, S.W.* 7)

We have previously shown (Fraenkel & Blewett, 1947) that mealworm larvae, when grown on an artificial diet, required, in addition to the then known B-vitamins, at least two B-factors, namely, folic acid and another factor, called by us tentatively B_T, which is contained in a charcoal filtrate from yeast or liver extract. In the absence of folic acid growth is greatly retarded, while without B_T the larvae die prematurely. Folic acid (pteroylglutamic acid) can be successfully replaced by vitamin B_c conjugate (pteroylheptaglutamic acid) which has, however, a slightly lesser activity. This is in line with the commonly accepted view that folic acid occurs in natural foods mainly in the form of the conjugate (Welch, Heinle, Nelson & Nelson, 1946). B_c conjugate, on the other hand, has no B_T activity.

Two commercial preparations of anti-pernicious anaemia liver concentrates, manufactured by Lederle Laboratories and Glaxo Laboratories respectively, have been found, in tests with *Tenebrio*, to be entirely devoid of folic acid activity, which indicates the virtual absence of both folic acid and B_c conjugate. These liver extracts also fail to show any B_T activity. This indicates that folic acid, anti-pernicious anaemia factor in liver concentrates and B_T are different substances.

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Some Observations on the Barritt Reaction for the Estimation of Creatine. By A. H. ENNOR and L. A. STOCKEN. (*Department of Biochemistry, Oxford*)

Eggleton, Elsden & Gough (1943) have stated that there are interfering substances present in liver which prevent the estimation of creatine by their modification of the Barritt reaction. Experiments have shown that this reaction is extremely sensitive to inhibition by thiol compounds, and that this inhibition is dependent upon the molar ratio of thiol to creatine. Thus it can be shown that approximately 50 % inhibition is produced by a molar ratio of cysteine ester:creatine of 0·6:1. Similar results are given by glutathione (GSH) and benzyl mercaptan.

Mere oxidation of the thiols is insufficient to remove the inhibitory effect because of the lability of the —S—S— bond in alkaline solution. The addition of *p*-chloromercuribenzoate has proved effective for the removal of the inhibition, and by this modification it is now possible to estimate creatine in trichloroacetic acid liver extracts. In view of the general distribution of GSH in tissues it is essential to add the mercury reagent to all extracts in which the molar ratio of thiol : creatine exceeds 0·2 : 1. In the case of small intestine, lung, kidney and spleen the addition has led to increases ranging from 60 to 250 %.

It has also been found that the Barritt reaction is inhibited by small amounts of Cu—12·6 μg. of Cu produces a 20 % inhibition in the estimation of 26·2 μg. of creatine. This inhibition may be quantitatively removed by the addition of KCN (0·01 M).

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The Effect of Some Thiol Compounds upon Trypsin, Chymotrypsin and Chymotrypsinogen. By R. A. PETERS and R. W. WAKELIN. (*Department of Biochemistry, Oxford*)

In 1943–4, we found that several thiol compounds inactivated crystalline trypsin and chymotrypsin (Kunitz & Northrop, 1935–6); this work was not reported in the stress of events. In the meanwhile, Grob (1946) has published work (also done in 1943) showing that thiol compounds inhibit the activity of crystalline trypsin.

In our experiments, the proteinase activity (as judged by liberation of non-protein nitrogen from casein solutions in 45 min. at 38°) was inhibited almost completely by a previous exposure of the enzymes to the following compounds at pH 7·6 in 0·1 m. concentrations: monothioethylene glycol (MTEG) cysteine (ester HCl) glutathione, BAL, sodium sulphide and *p*-thiosalicylic acid; no inhibition was produced by oxidized MTEG, cystine or pyrophosphate. MTEG and cysteine were also shown to have an action upon chymotrypsinogen. The action was more rapid to the alkaline side of pH 7·0. The thiol effect was irreversible. It was also shown with trypsin, acting upon benzyl arginine amide. Whereas none of the proteins decolorized

porphyrindin at pH 7·3, after thiol treatment and removal of thiol, best in acidified acetone (Stern & White, 1937), the proteins decolorized porphyrindin in an amount equivalent to 10–20 % of the total S considered to be present (Kunitz & Northrop, 1935–6; Brand & Kassell, 1941). This is interpreted as a scisson of an —SS— group present in the enzymes upon the lines of the well-known inactivation of insulin (Abel & Geiling, 1925; du Vigneaud, 1927); in this our view differs from that of Grob (1946). It is interesting that three proteins from the pancreas should behave similarly. The action of thiol compounds upon chymotrypsinogen leads to a rapid precipitation at pH 7·6.

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The Possible Importance of Copper in Soil Nitrification. By Howard Lees. (*Rothamsted Experimental Station, Herts*)

The effect of copper sulphate in soil nitrification has been studied in the soil percolation apparatus (Lees, 1947). At a percolate concentration of M/250, copper sulphate inhibited nitrification in three different soils, but at percolate concentrations of M/2000–4000 copper sulphate there appeared to be a faint stimulation. A nitrifying fen soil, treated with sodium diethyldithiocarbamate and thoroughly washed, lost its nitrifying power. This power was restored by the addition of M/250 or M/500 copper sulphate to the percolates but not by the addition of similar concentrations of zinc, cadmium, iron or manganese salts. These results agree with those previously obtained (Lees, 1946), and suggest that copper may be an important trace element in soil nitrification.

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Biosynthesis of the Penicillins. By E. Lester Smith and A. E. Bide. (*Glaxo Laboratories Ltd., Greenford, Middlesex*)

The now general use in penicillin production of phenylacetic acid or related compounds to increase the yield of penicillin G (II or benzyl penicillin) stems from research started independently in America and England. Delay in publication arises from wartime collaboration and secrecy agreements.

The discovery that addition of corn-steep liquor to a 'synthetic' medium induced formation of penicillin G rather than of F (I or Δ^2-pentenyl penicillin), suggested a biosynthetic precursor. DL-β-Phenyl-α-alanine and many of its degradation products were tested, usually in a 'synthetic' medium plus protein hydrolysate under surface culture conditions. β-Phenylethylamine, phenylacetamide, phenylacetic acid, *N*-(phenylacetyl) glycine and benzyl cyanide nearly doubled penicillin yields at optimal concentrations around 0·05 %. Other compounds such as phenylpyruvic acid, phenylacetic acid, β-phenylethyl alcohol and phenylacetaldehyde were less effective even at higher concentrations. Phenylalanine was quite effective but only at high concentrations, e.g. 1 %. The first group were about equally effective on purely synthetic media, and still useful on corn-steep media to supplement the small quantities of precursor present naturally. Several lines of evidence suggested that the phenomenon was due to partial biosynthesis of penicillin G, and not merely to the plant growth-promoting properties of phenylacetic acid and related substances. Phenylacetamide was shown to induce formation of (mainly) penicillin G on a synthetic medium that otherwise yielded penicillin F. Also β-(*p*-fluorophenyl)ethylamine yielded the corresponding *p*-fluorobenzylpenicillin.

The formation of small proportions of penicillin X (III or *p*-hydroxybenzylpenicillin) was presumably induced by a corresponding precursor present in corn-steep liquor. Penicillin X formation was markedly suppressed by both phenylacetic acid and phenylethylamine.

The use of supplements designed to induce biosynthesis of penicillins with aliphatic side-chains failed to increase the yield of total penicillin.

Many substances that might function as intermediates for other parts of the molecule, also had little or no effect on penicillin yields. More complex precursors such as *N*-(phenylacetyl)- and *N*-(β-phenylethyl)-amino-acids were used with the idea that they might be incorporated nearly intact into the penicillin molecule. Later evidence indicated that effective compounds were split by the mould, only the benzyl group appearing in the penicillin. A sulphur-free medium permitted only negligible mould growth. Out of a number of inorganic and organic sulphur compounds, sulphate was most effective in stimulating growth and penicillin production.

Penicillin Precursors in Corn-steep Liquor. By T. H. MEAD and M. V. STACK. (*The British Drug Houses Ltd., London*)

It was discovered by American workers that the addition of about 8 % of corn-steep liquor (C.S.L.) to the medium on which *Penicillium notatum* is grown causes a fourfold increase in the yield of penicillin and a reduction in the necessary period of incubation. In view of the consequent importance of C.S.L. and because no other substance having similar properties was known, this investigation was undertaken in 1944 as part of the penicillin research programme of the Therapeutic Research Corporation.

A study of the effects of adding to simple media, C.S.L. and fractions prepared therefrom indicated that the activity of the liquor is due to (i) mould growth stimulants, (ii) specific penicillinogenic factors. Difficulties encountered in assaying the specific factors in fractions of C.S.L. from which the non-specific growth stimulants had been removed, were overcome by using negative control cultures grown on a basic medium containing casein hydrolysate as growth promotor and by adding sufficient casein hydrolysate to the experimental cultures to bring their total nitrogen concentration up to those of the negative controls and of positive controls containing C.S.L.

With the aid of this assay it was shown that the specific penicillinogens of C.S.L. migrate to the cathode compartment on electrodialysis, are lost by evaporation of alkaline but not acid solutions, and form insoluble phosphotungstates from which they are recoverable in the usual manner. Since many benzyl compounds are effective penicillinogens (Lester Smith & Bide, 1948), the presence of β-phenylethylamine was suspected. This base was finally isolated from C.S.L., made alkaline with K_2CO_3, by vacuum-steam distillation. It was purified by ether extraction and recovery from the ether by aqueous acid and was separated from much accompanying ammonia by extraction of the mixed hydrochlorides with chloroform. Phenylethylamine hydrochloride was identified by analysis and mixed melting-point and conversion to the benzoyl derivative. The yield, probably far less than quantitative, was 0·017 %—sufficient to provide the benzyl component of 75 units of penicillin/ml. assuming the medium to contain 10 % of C.S.L.

β-*p*-Hydroxyphenylethylamine was isolated in 0·018 % yield by electrodialysis followed by solvent extraction. Its identity was established by analysis and mixed melting-point of the hydrochloride, free base and dibenzoyl derivative.

Extracts of unfermented maize were inactive. The bases isolated may arise from bacterial decarboxylation of the corresponding amino-acids during the steeping process. Changes in the bacterial population of the steeping vats may account for some of the variations in penicillinogenic activity displayed by different batches of C.S.L.

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The Biosynthesis of Penicillins by *Penicillium notatum*. By H. R. V. ARNSTEIN, J. R. CATCH and A. H. COOK. (*Organic Chemistry Department, Imperial College, London, S.W.* 7)

The increase in penicillin production caused by adding certain supplements to the nutrient medium on which *P. notatum* was cultured suggested that these supplements might be incorporated directly into the resulting penicillin. Accordingly, a strain was selected and grown under conditions which, in absence of the chosen supplement (phenylacetamide), afforded mainly Δ^2-pentenylpenicillin (penicillin I or F) and no significant yield of benzylpenicillin (penicillin II or G). Chromatography of the material obtained on the supplemented medium then showed it to contain a considerable proportion of

benzylpenicillin, the identity of which was left in no doubt by its conversion into the characteristic benzylpenillic acid.

A perhaps more stringent examination of the overall role of the supplement was provided by the chromatographic analysis of penicillin produced by a strain of *P. notatum* in presence of *p*-hydroxyphenylacetic acid. This penicillin mixture was found by indubitable chemical means to contain *c.* 35 % of *p*-hydroxybenzylpenicillin (penicillin III or X) as compared with *c.* 5 % of this variety of the antibiotic when grown without the supplementary nutrient.

These biosyntheses demonstrated some measure of control over the nature as well as the gross amount of the penicillin produced. The possibility of biosynthesizing 'unnatural' penicillins was thus envisaged and has since been realized elsewhere.

The Effect of Adrenal Cortical Hormone *in vitro* on Carbohydrate Synthesis in Liver Slices. By CHIUNG-YUN CHIU and D. M. NEEDHAM. (*Biochemical Laboratory, University of Cambridge*)

The observation of Seckel (1940) that addition of adrenal cortical hormone (eschatin) *in vitro* causes inhibition of glycogenolysis in liver slices, has been confirmed. It was further found that if the experiments were done in nitrogen instead of in air the eschatin was without effect on glycogenolysis. This observation showed that the action of eschatin is on glycogen synthesis rather than on glycogenolysis, and in the present work conditions favourable to synthesis were used.

The experiments were carried out in Barcroft pots filled with oxygen, the slices (about 100 mg.) being suspended in 3 c.c. of bicarbonate Ringer's solution buffered with M/150 phosphate, pH 7·4. The concentration of eschatin (kindly supplied by Messrs Parke Davis without preservatives) giving maximal effect after 1 hr. incubation at 37° was 6 rat units in 3 c.c.

Some typical results are shown in the table.

Substrate	Glycogen (mg./g.) Initial	Final + eschatin	Final no eschatin
Rat liver slices			
0·45 % D-lactate	0·76	1·75	1·06
0·45 % Na-pyruvate	1·77	4·32	1·92
1 % glucose	—	—	—
0·4 % DL-alanine	—	—	—
Rabbit liver slices			
0·45 % D-lactate	—	—	—
0·45 % Na-pyruvate	—	—	—
1 % glucose	6·39	13·20	11·00
0·4 % DL-alanine	1·09	1·18	0·79

Since eschatin seems to favour glycogen formation from all the substrates tested it seems probable that it affects some common reaction such as one of those concerned in continual oxidative resynthesis of ATP. This is under investigation.

In experiments in oxygen with no added substrate, there was either glycogen disappearance or a small increase in the slices without eschatin. When eschatin was added the balance was turned in favour of synthesis; this was shown either by a decrease in glycogen disappearance or a greater increase in glycogen content. Since it seems likely that, in the absence of added substrate, protein is the source of the glycogen formed, the effect of eschatin on non-protein nitrogen formation was tested. About 20 % more non-protein nitrogen was found in the presence of eschatin in each case at the end of the experiment.

In four experiments on rat liver slices with pure desoxycorticosterone acetate (DOC) it was found that 10 μg. caused marked increase in glycogen content: for example (*a*) with pyruvate as substrate, initial value 1·31 mg./g., final with DOC 3·05; without DOC 1·37; (*b*) with D-lactate, initial value 1·34 mg./g., final value with DOC 1·85; without DOC 1·40.

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The Hexokinase-inhibiting Activity of Diabetogenic Pituitary Extracts. By E. REID (Carnegie Research Fellow), R. H. SMITH and F. G. YOUNG. (*Department of Biochemistry, University College, London*)

The important discovery of C. F. Cori and his colleagues (Price, Cori, C. F. & Colowick, 1945; Price, Slein, Colowick & Cori, G. T., 1946; Cori, C. F., 1946; Colowick, Cori, G. T. & Slein, 1947) that the transfer of phosphate from adenosine triphosphate to glucose under the influence of the enzyme hexokinase is inhibited *in vitro* by anterior pituitary extracts, and that this inhibition is counteracted by the addition of insulin to the system *in vitro*, has led many to the assumption that the pituitary substance concerned is the diabetogenic factor. The discoverers themselves do not claim that this is so, and from

their own demonstration of the extreme lability of the pituitary hexokinase-inhibiting factor it may be concluded that there is no simple identity established between this factor and the pituitary diabetogenic substance, since in our hands the latter remains stable at 0° for many weeks under suitable conditions and may even survive long periods at room temperature (Young, 1946).

In a number of instances our experiments have confirmed Cori's essential findings, including the reversal by insulin of pituitary hexokinase inhibition and the lability of the pituitary factor concerned. Furthermore, we have failed to find an insulin-reversible hexokinase inhibitory activity in many anterior pituitary preparations which are highly diabetogenic in the adult intact cat, including, in some instances, fractions prepared according to the methods of Cori *et al.* for the production of the pituitary hexokinase inhibition. These experiments have been carried out with pituitary extracts free from added antiseptic. In other pituitary preparations we have had present 0·01 % sodium ethyl mercurithiosalicylate ('Merthiolate'-Eli Lilly); these have often exhibited hexokinase inhibitory activity *in vitro* but in a manner which is not significantly reversible by insulin in our hands. Moreover, the factor here concerned appears to be stable at 0°.

Our results demonstrate a wide divergence between the insulin-reversible hexokinase inhibitory activity and the diabetogenic activity of anterior pituitary extracts. Nevertheless, in agreement with Cori, we have observed that when crude anterior pituitary extracts (in our experiments possessing high diabetogenic activity but no demonstrable insulin-reversible activity on hexokinase *in vitro*) are administered to intact rats the muscle hexokinase activity is influenced in such a way that it can be enhanced by the addition of insulin *in vitro*. It may well be that the insulin-reversible hexokinase inhibitor and the diabetogenic factor of anterior pituitary extracts are substantially the same but that in stored pituitary extracts the factor undergoes a rapid change which deprives it of its insulin-reversible inhibitory activity *in vitro*, such a change being reversible *in vivo*.

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The Hexokinase Activity of Retinal Extracts. By MARGARET KERLY. (*Department of Biochemistry, University College, London*)

The high glucolytic activity of aqueous extracts of ox retina suggested an investigation of their hexokinase activity. Extracts were prepared according to our previous technique (Kerly & Bourne, 1940); they contained negligible amounts of glucose or of adenosine triphosphate (ATP). Hexokinase activity was tested by incubation in a bicarbonate buffer containing Mg^{++}, NaF and an excess of glucose and of ATP. In crude extracts, even in the presence of a high concentration of fluoride, glucose-6-phosphate formed by the reaction of glucose and ATP undergoes further change. Most of the labile phosphate transferred from the ATP accumulates as an ester not readily hydrolyzed by 1·0 N-HCl, but an appreciable amount of an easily hydrolyzable ester is also formed. This complicates estimation of the hexokinase activity either by determination of loss of ATP or by manometric measurement, and it is preferable to determine glucose utilization. When 0·1 ml. extract is incubated at 30° for 10 min. about 200 μg. glucose is removed. In these conditions the rate of the reaction is nearly linear and varies with the amount of extract added. Fructose is utilized at a rather more rapid rate; neither 3-methylglucose nor galactose is attacked.

These extracts retain their hexokinase activity for several days when stored in the cold room. Dialysis for short periods (up to 24 hr.) has little effect on activity, but after 72 hr. activity is slight.

Preliminary attempts at purification using the methods of Cori & Slein (1947) have shown that an active extract can be recovered from precipitates formed on addition of acetone or ammonium sulphate to crude extracts.

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The Effect of Adrenaline Injection on Glucose Utilization by Muscle. By J. A. COHEN and DOROTHY M. NEEDHAM. (*School of Biochemistry, University of Cambridge*)

The possibility that the hyperglycaemia following adrenaline administration might be partly due to a decrease in peripheral glucose utilization has been the subject of controversy. The experimental evidence was all derived from intact or eviscerated animals, and one of us (1947) has recently tried to throw some light on the problem by experiments *in vitro*. It was shown that injection of adrenaline into rats did lead to a partial suppression of the anaerobic glycolysis in diaphragm. The results suggested that this effect was an indirect one, mediated by other hormones possibly of hypophyseal or adrenal cortical origin.

In the present series of experiments we have tried to elucidate this effect on muscle by investigating the glucose utilization of fresh rat muscle extracts. Such extracts from normal animals, under our conditions (0·2 % glucose, 0·007 M-ATP) utilize glucose in the course of 40 min. at 30° to the extent of about 2·3 mg./ml. extract. Extracts made in exactly similar manner from litter mates injected 45 min. previously with a dose of adrenaline (1 mg./kg.) showed a glucose utilization decreased by 29 %. These results are the average from experiments on 19 normal and 19 adrenaline injected animals.

This inhibition was also found in experiments of short duration (10 min.). In these, the glucose concentration was 0·05 % and the ATP concentration 0·005 M. The glucose utilization under these conditions was about 0·7 mg./ml. extract in the extracts from normal animals and 28 % less in the extracts from injected animals. Thirty animals were used in each case for obtaining these averages. By adding fluoride to the system (0·06 M) the ATP can be maintained constant in the short-term experiments (since ATP-ase is inhibited) and under these conditions the glucose utilization may be regarded as a measure of hexokinase activity. An inhibition of 13 % was found, suggesting that at least part of the inhibition in the absence of fluoride is due to an effect on hexokinase. As in the case of diaphragm, it was not possible to show any direct action of adrenaline on muscle extract; this indicates an indirect action, e.g. through other hormones liberated as a result of adrenaline injection or through products resulting from adrenaline metabolism. One of these products, adrenochrome, was found not to have any inhibitory effect in short term experiments.

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The β-Glucuronidase Activity of Ox Spleen. By G. T. MILLS. (*Biochemistry Department, St Thomas's Hospital Medical School, London, S.E.* 1)

The data of previous workers on β-glucuronidase (Masamune, 1934; Oshima, 1936; Fishman, 1939; Graham, 1946; Talalay, Fishman & Huggins, 1946) indicated the presence of only one enzyme in spleen and kidney which hydrolyzed β-glucuronides. The pH optima for these preparations have been stated to be 5·0–5·3 for L-menthylglucuronide (Oshima, 1936; Fishman, 1939), 5·3–5·6 for phenylglucuronide (Oshima, 1936) and 4·5 for phenolphthalein glucuronide (Talalay *et al.* 1946).

A reinvestigation of the preparation and purification of β-glucuronidase, involving the application of the variable solvent solubility test, has shown the presence in ox spleen of two enzymes hydrolyzing certain β-glucuronides. These two β-glucuronidases have been separated and purified by ammonium sulphate fractionation and examined separately.

The pH optima for the hydrolysis of L-menthylglucuronide are 4·5 and 5·0 for these preparations.

The preparative methods of previous workers were such as to eliminate the enzyme having the lower optimum pH.

Data are presented on the hydrolysis of β-glucuronides other than D-menthylglucuronide by the two separate enzymes.

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Pathologic Effects of Ionizing Radiations and Radioactive Materials. By A. M. BRUES (introduced by Sir E. MELLANBY). (*Argonne National Laboratory, Chicago, Illinois*)

Certain purely chemical effects of ionizing radiations are understood. These include the dissociation of water into H and OH radicals and the inhibition of certain enzymes such as those containing sulphydryl groups (Dale, 1942; Barron, 1947). In addition to these proximate effects, various forms of genetic and cell damage have been extensively studied. The relation of these changes to most clinical radiation effects remains largely to be established.

In acute lethal radiation exposure in mammals, we see an immediate response corresponding to clinical X-ray sickness. Susceptible animals will show hypotension, vomiting, or erythema of the skin (Painter, Prosser & Moore, 1947). By the second day, acute symptoms have largely abated, but there is a marked decrease in circulating white cells which continues until death. During the ensuing week, animals refuse food and water and lose weight. There is increasing anaemia, increased plasma and extracellular volume, accelerated sedimentation rate, increased renal excretion of phenol red, and decreased levels of blood non-protein nitrogen, urea and polypeptides. Histologic damage is most marked in the blood-forming organs, gonads, and intestinal tract.

During the second week, the clotting time is greatly increased due to an overabundance of circulating heparin (Allen & Jacobson, 1947); fever and subcutaneous oedema appear and death occurs, attended by loss of blood, bleeding into vital areas, such as the heart and central nervous system, ulceration of mucous membranes and a terminal shock-like state. A shift in the plasma protein pattern (increased beta and gamma globulin) has been seen in dogs, but is not specific for radiation sickness (Prosser, Painter & Swift, 1947).

Later effects in surviving animals include anaemia, lymphoid atrophy, and in mice, an enhanced tendency to lymphoma. Chronic effects include decrease in life span, greying of hair and an increased tumour incidence.

Acute effects of absorbed radio elements generally resemble those of total body radiation. Their late effects depend upon localization of the element and, as in the case of radium, a great increase in bone tumours is seen after absorption of radiostrontium, as well as plutonium and many of the radioactive rare earths (Lisco, Finkel & Brues, 1947).

Since C^{14} has been shown to deposit in bone (Bloom, Curtis & McLean, 1947), the question is raised whether it may present a human hazard analogous to radium. An attempt has been made to consider the human tolerance on the basis of known facts and it is presently thought that a total human absorption in excess of 30 microcuries must be considered hazardous until further knowledge is gained regarding the retention of carbon administered in various forms. In the case of a hypothetical carbon compound, such as a steroid, which might remain localized in a very small organ, even this figure may need revision.

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DEMONSTRATIONS

A Simple Automatic Soil Percolator. By HOWARD LEES

An apparatus that continually percolates a column of soil with small quantities of metabolite solution from a reservoir, to which they are returned after percolation. The apparatus, which has already been described (Lees, 1947), is a modified and improved version of my original one (Lees & Quastel, 1944). Any weight of soil up to 40 g. of any percolate volume between 50 and 200 ml. may be used. The apparatus is driven equally well by air pressure or by suction, is in a large measure self-adjusting in operation, is quickly assembled or disassembled, and is simply constructed, there being only two glass joints. At no point in it does the soil or percolate touch any material save glass. The soil cannot become waterlogged because if air flow through the soil becomes impeded, percolation automatically stops.

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An Apparatus for the Preparative Electrophoretic Separation of Amino-acids, Peptides and Proteins. By J. A. V. BUTLER and JOAN M. L. STEPHEN

A Simple Apparatus for the Extraction of Corticosteroid-like Substances from Urine. By ALICE M. ROBINSON and F. L. WARREN. (*Pathology Department, St Bartholomew's Hospital, E.C.* 1, *and Physiology Department, St Mary's Hospital Medical School, London, W.* 2)

Several procedures have been devised for the assay of 'corticosteroid-like' substances in human urine by chemical methods. The initial step usually consists in the extraction of urine (either fresh or after acidification) with chloroform. Normal methods of extraction by hand-shaking in separatory funnels frequently lead to troublesome emulsions and partial loss.

The formation of these emulsions is avoided by the exclusion of air during admixture of the two liquids. In the present type of apparatus this is achieved by delivering the urine in the form of a fine spray at the bottom of a column of chloroform. The urine rises through the chloroform in droplet form and coalesces as an upper aqueous layer which is removed via a side outlet and recirculated through the chloroform.

In an extraction apparatus of appropriate size the whole of the 'corticosteroid-like' substances in 100 ml. of urine can be transferred to chloroform in 50–70 min. (ten recirculations) and this amount of extract, after further purification, is sufficient for chemical assay.

Apart from the ease and semi-automatic nature of the extraction, a further advantage is the increased speed (compared with hand-extraction) which is an important factor in obtaining maximum yields in view of the instability of the 'corticosteroid-like' substances.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **262nd Meeting** *of the Biochemical Society was held in the London School of Hygiene, Keppel Street, London, W.C.* 1, *on Saturday,* 13 *December* 1947 *at* 11 *a.m., and took the form of a Symposium on 'The Biochemical Reactions of Chemical Warfare Agents'.*

The Reactions of Mustard Gas in Aqueous Solution.

By A. G. Ogston. (*Department of Biochemistry, Oxford*)

An effort is made to trace the history of research carried out during the years 1939–45 on the replacement reactions of mustard gas in aqueous solution, in Britain and in the U.S.A. and to consider the results in relation to the actions of mustard gas on biological systems. Starting from the pioneer kinetic work of Peters & Walker (1923), kinetic investigation showed that the reaction of mustard gas is of the 'SN_1' type described by Ingold: an initial first-order reaction gives rise to an activated form of mustard gas, which then undergoes second-order reactions with a wide variety of reagents. These secondary reactions compete with each other, so that the final products depend on the relative concentrations and velocity constants of reaction of the substituting reagents present in the system. Later work filled in and refined this simple account, taking into consideration the effects on the kinetics of reaction of the presence of two reactive chlorine atoms in mustard gas, of varying ionic strength and of the reversibility of formation of some of the products of reaction, particularly those involving the formation of sulphonium compounds between mustard gas and other sulphides.

It appeared from these investigations that mustard gas can combine with all the chemically reactive groups found in biological systems. Direct investigations, especially on proteins and enzymes, gave results in full accord. Certain remaining problems concerning the toxic effects of mustard gas are discussed.

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Reactions of Mustard Gas with Enzymes in Tissues and *in vitro*.

By D. M. Needham. (*Biochemical Laboratory, University of Cambridge*)

Mustard gas (*H*) is not a general enzyme poison. Out of some 30 enzymes examined only those of two well-defined groups are markedly inhibited—the phosphokinases and closely related enzymes and a small group of proteinases.

Amongst the phosphokinases, hexokinase was the first to be examined and found to be *H*-sensitive. It was shown to be an —SH enzyme, but the inhibitory effect of *H* is not through the —SH group. Pure crystalline hexokinase was prepared from yeast in several laboratories; its reaction with *H* containing radioactive sulphur was examined by Bailey and Webb, who came to the conclusion that only 5–6 molecules of *H* are necessary per molecule of hexokinase for complete inactivation.

It was soon found that other phosphokinases are *H*-sensitive, e.g. creatine-, phosphopyruvic-, and glycerol phosphokinases and deuterohexokinase. The phosphorylation connected with oxidations in extracts is also inhibited by *H*, although the oxygen uptake is not.

The proteinases inhibited by *H* are pure pepsin (Northrop), a new tissue peptidase (Bergmann) and a new skin proteinase (Peters).

The work concerned with the effect of *H* on enzymes in tissues has dealt mainly with skin, marrow, intestinal and gastric mucosa and muscle. In this field it is often necessary to consider whether the effects observed are due to the action of *H* itself or to the action of products formed in the tissues.

Some Reactions of Mustard Gas ($\beta\beta'$-Dichlorodiethyl sulphide) with Proteins. By J. C. BOURSNELL. (*School of Biochemistry, University of Cambridge*)

In this paper a short account was given of the action at room temperature and at 37°, of mustard gas (*H*) and some of its derivatives upon a variety of proteins.

Some indication was given of the extent and firmness of the combination which takes place, and also of some of the groups in the protein molecule which are most probably attacked. In a number of these experiments use was made of radioactive sulphur (S^{35}) as a tracer element.

Attention was also given to some immunological investigations using the reactions of mustard gas (and some of its derivatives) with serum proteins to produce protein derivatives with a new immunological specificity; and reference was made to the inactivating action of mustard gas and some of its derivatives upon complement.

The Development of British Anti-lewisite. By R. H. S. THOMPSON. (*Department of Chemical Pathology, Guy's Hospital Medical School*)

The view that the toxic action of arsenoxides on living cells involves a reaction with thiol groups is not new. Voegtlin and his colleagues (1923, 1925) and Peters & Walker (1924) had each demonstrated the limited ability of certain monothiols to cause some degree of protection of certain biological systems from intoxication by therapeutic and other arsenicals. The effects obtained were, however, much less complete when exerted against sodium arsenite, and when, during the last war, monothiols were tested against lewisite no significant degree of protection could be demonstrated.

A re-examination of the problem indicated that the mode of action of small concentrations of arsenic is selective. Of a number of different enzymes examined the pyruvate oxidase system was shown to be the most sensitive, and both *in vitro* and *in vivo* evidence was obtained suggesting that the first point of attack of arsenicals is on pyruvate oxidation. In their actions on enzymes arsenicals in fact resemble iodoacetate, a known reactor with thiol groups.

To study this reaction further, arsenical derivatives of kerateine were prepared, analysis of which showed that arsenic was present largely in the proportion of 1 atom of arsenic to 2 SH groups in the original kerateine. In the hope of forming a ring compound with the arsenical at least as stable as that formed with the tissue 'acceptor' dithiol compounds were therefore studied as potential antidotes. 2,3-Dimercaptopropanol (British Anti-Lewisite) was synthesized (Stocken, 1947) and found to be an efficient antidote when tested *in vitro* with the pyruvate oxidase enzyme system, and *in vivo* against lewisite intoxication or contamination in rats, guinea-pigs and man (Stocken & Thompson, 1946).

From this work with arsenicals and thiol antidotes, evidence has been obtained in favour of the view that, in addition to 'monothiol' enzymes, 'dithiol' enzymes may exist, which, like the pyruvate oxidase system, require the presence within their molecule of 2 closely placed SH groups.

Reactions of Lachrymators with Enzymes and Proteins. By M. DIXON. (*School of Biochemistry, University of Cambridge*)

A survey of the action of lachrymators on enzymes (Mackworth, 1940–1) showed that they act as powerful poisons of —SH enzymes. All the lachrymators, whatever their chemical structure, share this common property. All the —SH enzymes are poisoned by them; other enzymes are not affected, nor are the oxidized forms of the —SH enzymes. This suggested that they react specifically with —SH groups in proteins, and direct —SH tests (Morgan, 1942) confirmed this. Masking the —SH groups by reversible combination with arsenicals protects the enzyme against attack by lachrymators (van Heyningen, 1942). The lachrymators form valuable specific reagents for identifying —SH enzymes.

The '—SH theory' provides for the first time an explanation for the existence of two main groups of lachrymators: (*a*) substances containing a halogen atom in some such group as $—CH_2Cl$, together with a neighbouring keto or other group which makes the halogen 'positive', (*b*) substances containing a —CH=CH— group, also with a neighbouring keto or other group which polarizes the double bond. Both these types of molecules readily combine with —SH groups.

Lachrymators of type (*a*) can be used for manometric estimation of —SH groups in proteins. With heat-denatured ovalbumin the values obtained agree with those given by other methods, indicating that the protein contains no other groups which react with the lachrymator. Native ovalbumin gives no immediate reaction, but a slow reaction occurs, which ultimately reaches about the same value and is probably due to a slow production of —SH groups. The product, however, does not appear to be denatured.

The Reaction of Fluorophosphonates with Esterases. By E. C. Webb. (*Biochemical Laboratory, University of Cambridge*)

The early studies on the fluorophosphonates prepared by Dr B. C. Saunders had shown that they were not only highly toxic, but showed marked sympatheticomimetic actions in sub-lethal concentration, similar (except in time relations) to those produced by the alkaloid eserine. It was demonstrated independently in 1941 by Adrian & Feldberg and by Mackworth, that serum cholinesterase is inhibited by alkyl fluorophosphonates, as well as by eserine.

The inhibition by eserine reaches a maximum within a few minutes, can be reversed by dialysis, and shows substrate competition; while that by fluorophosphonate is non-competitive and cannot be reversed by dialysis. The time course of inhibition by fluorophosphonate shows two stages. Recently, Nachmansohn, Rothenberg & Feld (1947) have shown that this inhibition can be partially reversed by dilution in the early stages.

Horse serum cholinesterase is 50% inhibited in 15 min. at 20° under standard conditions by $1{\cdot}3 \times 10^{-9}$M-di*iso*propyl fluorophosphonate (the most active ester examined); the inhibition is complete at 10^{-8}M, and still measurable at 10^{-11}M. The figures, taken in conjunction with the absolute activities obtained by Easson & Stedman (1936) and Straus & Goldstein (1943), suggest that complete inactivation can be produced by the presence of 1–3 molecules of fluorophosphonate/enzyme active centre.

Certain other esterases, especially liver esterase and human milk lipase, are inhibited by fluorophosphonate. The pattern of sensitivity to fluorophosphonate of a range of esterases is different from that to eserine or to fluoride, but similar to the pattern of sensitivity to tri-*o*-cresyl phosphate and tri-*o*-chlorophenyl phosphate described by Bloch (1943) and Hottinger & Bloch (1943).

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Pharmacology of Chlorethylamines. By E. Boyland. (*The Chester Beatty Research Institute, The Royal Cancer Hospital (Free), London, S.W. 3*)

Simple aliphatic chlorethylamines with more than one chlorethyl group are vesicants and many of their pathological effects are similar to those produced by vesicants containing sulphur or arsenic. Compounds with only one chlorethyl group such as dimethyl β-chlorethylamine are much less toxic but have a prolonged neurotoxic action causing tremor and incoordination of movement. Mice treated with such compounds resemble waltzing mice in their behaviour. Dibenzylchlorethylamine blocks and reverses the vasopressor response to adrenaline.

The bis-(β-chloroethyl)-amines with simple alkyl groups, the tris-(β-chloroethyl)-amines and the simple tetrakis-(β-chloroethyl)-diamines are radiomimetic poisons like mustard gas. The effects which they produce are similar to those following treatment with X-rays or radioactive materials. These effects include (1) inhibition of mitosis and fragmentation of chromosomes in plants and animals, (2) mutations in *Drosophila*, *Neurospora* and *Escherichia coli*, (3) nausea and vomiting in human subjects, (4) greying of hair, (5) reduction of the concentration of leucocytes and thrombocytes in the blood, (6) haemoconcentration in dogs, and (7) death of animals after 6 to 10 days. Poly-(β-chloroethyl)-amines have been used in place of radiation therapy in treatment of leukaemia, Hodgkin's disease and bronchogenic carcinoma.

The poly-(β-chlorethyl)-amines react rapidly with water in neutral solution to give chlorethyl ethylene imines. These compounds react further to give hydroxyethyl ethylene imines which are toxic substances killing animals very rapidly following subcutaneous injection.

The **263rd Meeting** *of the Biochemical Society was held at the British Postgraduate Medical School, Ducane Road, Hammersmith, London, W. 12, on Saturday, 24 January* 1948, *at* 11 *a.m., when the following papers were read:*

COMMUNICATIONS

The Micro-estimation of Amino-nitrogen and its Application to Paper Partition Chromatography. By A. J. Woiwod. (*Wellcome Physiological Research Laboratories, Beckenham, Kent*)

A copper phosphate reagent has been developed for determining 1–30 μg. α-amino-N, based on the copper reagent of Pope & Stevens (1939). The amino compound reacts with the copper phosphate to give a soluble copper complex which is quantitatively decomposed by sodium diethyl-dithio-carbamate. The yellow colour formed is extracted into amyl alcohol, and its optical density determined with the Spekker absorptiometer. The amount of copper may be related to α-amino-N by the use of standard curves. The ratio α-amino-N/copper for a number of amino-acids and related compounds is given below. For the formation of the complex A_2Cu the theoretical ratio should be 0·44.

To prepare the reagent, 0·16 M-$CuCl_2 . 2H_2O$ (1 vol.) is added to Na_3PO_4 solution (1 vol.) prepared as described by Pope & Stevens (1939). The copper phosphate sludge formed (1 vol.) is then refluxed for 30 min. with 0·18 M-$Na_2HPO_4 . 12H_2O$ (2 vols.). This reagent is stable for some weeks. The amino-acid solution is pipetted into 0·18 M-$Na_2HPO_4 . 12H_2O$ containing $Na_2B_4O_7 . 10H_2O$ (4·0 g./l.) and an equal volume of the copper phosphate reagent added. After standing 30 min. at 20° C. the mixture is filtered and the copper in the filtrate estimated absorptiometrically.

The method has been successfully applied to the quantitative estimation of amino-acids separated by paper partition chromatography (Consden, Gordon & Martin, 1944).

	Ratio $\frac{\alpha\text{-amino-N}}{\text{Cu}}$		Ratio $\frac{\alpha\text{-amino-N}}{\text{Cu}}$
Alanyl-glycine	0·22	Tyrosine	0·43
Histidine	0·25	Arginine	0·44
Hydroxyproline	0·38	Phenylalanine	0·45
Aspartic acid	0·39	Cystine	0·45
Serine	0·40	Tryptophan	0·46
Threonine	0·40	Glutamine	0·46
Valine	0·41	Alanine	0·46
Leucine	0·41	Proline	0·48
*iso*Leucine	0·41	Lysine	0·52
Glutamic acid	0·42	Ornithine	0·58
Methionine	0·42	Glucosamine	1·11
Asparagine	0·43	β-Alanine	6·89
Glycine	0·43		

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The Inhibition of Acid Phosphatase by D-Tartrate. By M. A. M. Abul-Fadl and E. J. King. (*Postgraduate Medical School of London, W.* 12)

Amongst the organic substances examined by Kutscher & Wörner (1936), alcohols, especially the lower aliphatic homologues and certain narcotics, were found to exhibit strong inhibitory action on the prostate enzyme. King, Wood & Delory (1945) also pointed out that the red cell phosphatase was destroyed by ethanol. The effect of formaldehyde has been discussed by the authors in a previous communication (1947). The effect of certain organic acids has now been investigated with regard to their action on normal plasma, prostate, and red cell acid phosphatases. Oxalates were found to inhibit all of them, nearly to the same extent. Bile acids inhibited almost equally the red cell and prostate enzymes, but did not affect the acid phosphatase of normal plasma.

Tartrates were without noticeable effect on either the plasma or the second (pH 5) red cell phosphatase, but, on the other hand, were very strongly inhibitory toward the prostate enzyme. It was thought that the chemical configuration of this acid might be involved; and a variety of related hydroxy and other carboxylic acids were examined (formic, acetic, lactic, pyruvic, malic, succinic, citric and salicylic acids). None of these was found to exhibit the effect shown so strongly by tartaric. With the different

stereo-isomerides of tartaric acid, the property of inhibition was only possessed by the D- and DL-forms, while the L- and the *meso*-isomerides were ineffective. Thus the tartrate inhibition can be considered to be stereomeric. It sharply distinguishes the plasma and red cell phosphatase on the one hand, from the prostate and other tissue phosphatases on the other.

Like the competitive type of inhibition to which the oxalate and possibly the fluoride inhibitions belong, this D-tartrate inhibition can be reversed by dialysis. It is distinct from the destructive type of inhibitor, e.g. by ethanol and formaldehyde.

The effect of D-tartrate on the other tissue phosphatases at various pH values was also studied. It has been found that the liver, kidney and spleen enzymes are also strongly inhibited in the acid range, while not affected in the alkaline. The red cell phosphatases were inhibited in the acid range of pH 3·8–4·6, while from pH 4·8 upwards there was either no inhibition or slight activation. This favours the presence of two acid phosphatases in erythrocytes, as found by Roche, Thoai & Baudoin (1942), and confirmed by ourselves.

The application of tartrate in the determination of serum acid phosphatase for clinical purposes is being investigated.

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Bilirubin: Modified Procedure without Precipitation of Proteins. By R. V. Coxon and E. J. King

The Apparent Arginine Content of Human Plasma Proteins by the Sakaguchi Reaction. By J. W. Keyser

A New Study of Protein Digestion (in Dogs). By C. E. Dent* and J. A. Schilling. (*Departments of Pathology and of Surgery, University of Rochester, New York*)

Doubt still exists as to whether the substances absorbed into the portal blood during protein digestion are mainly amino-acids or peptides (Verzar & McDougall, 1936). The existing evidence suggests that absorption of all, even also of traces of intact protein, may occur to some extent.

The paper chromatographic technique (Consden, Gordon & Martin, 1944) has now been applied to the problem. Some 60 amino-acids or closely related compounds likely to occur in nature have been studied on the chromatograms and the method is confirmed to be highly specific qualitatively and also to allow of rough quantitative comparisons. Portal blood, drawn by means of the London cannula (London, 1936), and in some cases also jugular blood and urine, have been examined at 1, 2·5 and 5 hr. intervals after feeding various proteins. In nearly every case the dog was previously submitted to a partial gastrectomy operation. This enabled the protein to pass more rapidly from the stomach into the small intestine. The ensuing quickened digestion resulted in much larger rises (4–6 times normal) of the levels of the metabolites in the portal blood, and the peak of absorption was always within the 5 hr. span of the experiment. The plasma proteins were removed by ultrafiltration before the chromatographic analysis.

It has been shown in this way that casein, casein hydrolysate (Amigen), ground beef and human serum albumin are all absorbed mainly, if not entirely, as free amino-acids. No peptides were found in the blood although some samples showed, after acid hydrolysis, slight rises in certain amino-acids. The urine after feeding human albumin did, however, show the presence of 'peptides', i.e. ninhydrin-reacting substances, moving characteristically on the chromatograms and readily decomposable by hydrolysis.

In marked contrast to the above results, there were no appreciable changes in blood amino-acid

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levels after feeding dog's plasma protein although the protein was shown to have disappeared from the gut. There was also a tendency to diarrhœa and vomiting in these dogs. This was not observed with the other proteins.

The changes in free and bound plasma amino-N in several of the experiments have been kindly checked by Dr H. N. Christensen, using the ninhydrin-CO_2 method.

The suggestion is made that the dog's plasma protein was absorbed intact under the conditions of the experiment. The dog's intestinal mucous membrane appears therefore to be able to distinguish its own plasma proteins from a human plasma protein as well as from the beef and casein. This interpretation of the facts leads, however, to serious difficulties in reconciling other work in which homologous plasma was fed.

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The Relative Importance of Antibodies and Vitamin A in Preventing Disease in Young Calves. By F. Blakemore, A. W. Davies, Eva Eylenburg, T. Moore, K. C. Sellers and A. N. Worden. (*Institute of Animal Pathology and Dunn Nutritional Laboratory, Cambridge*)

Exploratory experiments confirmed previous claims that calves develop fatal 'white scour' when they are denied colostrum and kept on a diet of whole or separated milk. Ayrshire and Jersey calves were found to be particularly susceptible to the disease, while Friesians, which are larger in size, were more resistant.

The protective action of colostrum has been ascribed to its antibody content, but since the calf is normally born with low reserves of vitamin A its significance as a rich source of this vitamin has also been considered. Attempts to protect calves from 'white scour' with concentrated preparations of vitamin A, administered in various forms, were unsuccessful. If the vitamin was given before 'white scour' had started its absorption was indicated by a temporary rise in the level in the plasma, and by the recovery of a portion of the vitamin from the liver *post-mortem*. If dosing was commenced or continued after the development of 'white scour', however, there was no corresponding rise in the level in the plasma, and much vitamin A was excreted in the faeces. On the other hand, calves were kept alive by feeding them on colostrum followed by separated milk. Evidence was obtained that the protective value of colostrum was associated with its globulin content, since calves inoculated with precolostrum, and fed on separated milk, survived the early critical period. Antibodies, in a concentrated form, were demonstrated in precolostrum.

The possibility that deficiency of vitamin A may at a later stage be of practical importance in the rearing of bovines in this country was suggested by the constriction of the optic nerves found in steers fed upon diets consisting largely of sugar-beet pulp. Analysis of the blood and livers of some of the animals afflicted with this disease indicated that they were deficient in vitamin A. Other affected animals had adequate reserves of vitamin A when killed, but their dietary history usually suggested the possibility of deficiency during a critical period of their development.

The Nutritive Value for the Calf of Colostrum and its Fractions. By R. Aschaffenburg, S. Bartlett, S. K. Kon, P. Terry, S. Y. Thompson and D. M. Walker (*National Institute for Research in Dairying, University of Reading*), C. Briggs and E. Cotchin. (*Research Institute of Animal Pathology, Royal Veterinary College*)

It is uncertain which is the fraction that makes colostrum so valuable to the new-born calf. We have, therefore, separated with a Sharples supercentrifuge, into a fatty and a non-fatty fraction, Ayrshire and Shorthorn colostrum collected during the first 48 hr. after calving. The fatty fraction contained *c.* 75% fat and it was later found desirable to prepare a clarified fat which did not contain any non-fatty material. The fatty fractions were homogenized with reconstituted dried skim milk to a content of *c.* 3·5% fat, while non-vitaminized margarine was dispersed in the non-fatty fraction to the extent of *c.* 2% fat. We also made diets of untreated colostrum, of reconstituted colostrum

(separated colostral fat rehomogenized with the non-fatty fraction) and of a 'synthetic' colostrum consisting of the reconstituted milk (as described below) supplemented with 52 mg. of carotene and 150,000 i.u. of vitamin A. All diets were stored at −25° in portions of 300 ml. in waxed carton containers and samples from several cows were bulked to provide 16 lb. to be fed over the first 2 days of life to Ayrshire and Shorthorn calves which had had no access to colostrum. Subsequently, the calves received a reconstituted milk mixture (1 lb. dried skim milk, 4 oz. glucose, 3 oz. non-vitaminized margarine, made up to 10 lb. with water) plus 3500 i.u. vitamin A and 700 i.u. vitamin D daily. Those calves given the 'synthetic' colostrum received also 500 mg. of vitamin C and 50 mg. of nicotinic acid daily for the first 10 days of life. Blood samples at birth and blood and liver samples at slaughter were taken for the measurement of vitamin A and carotene. The performance of the calves was assessed over 5 (later reduced to 3) weeks on general health, weight increase, body temperature and occurrence of scours. The maximum performance score was 10, graded down to 0 for a calf which died. The results for a total of 66 calves (32 Shorthorns, 34 Ayrshires) are summarized in the table.

Treatment ...	1	1A	2
	Crude fatty fraction	Clarified fatty fraction	Non-fatty fraction
Number of calves:			
Used	8	8	16
Died	1	4	1
Performance score*	62	34	81
Treatment ...	3	3A	4
	Reconstituted colostrum	Stored untreated colostrum	'Synthetic' colostrum
Number of calves:			
Used	16	11	7
Died	4	—	5
Performance score*	68	92	20

* $\frac{\Sigma \text{ Actual scores} \times 100}{\Sigma \text{ Possible scores}}$.

Treatment 2 is clearly superior to treatment 1A and the good results evident from treatment 1 indicate that the essential factor in the aqueous phase is active in small concentrations. The four deaths which occurred on treatment 3 remain unexplained. The 'synthetic' colostrum was of little value to the calves. *Post-mortem* examination showed that of the 15 calves which died 13 had fatal scours. Vitamin A reserves at 5 weeks proved unrelated to the initial intake. However, in calves which died after having scoured profusely the reserves of vitamin A were low at death.

Observations on the Vitamin C Metabolism of the Piglet. By R. Braude, S. K. Kon and J. W. G. Porter. (*National Institute for Research in Dairying, University of Reading*)

Recent work by Braude, Kon & Thompson (1946) and Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (1947) has shown that both sow's colostrum and milk are appreciably richer in ascorbic acid than those of most other species. It seemed of interest, therefore, to investigate whether the piglet, unlike the adult pig, is dependent on an exogenous source of vitamin C.

The vitamin C in sow's colostrum, goat's milk and in a mixture of goat's and cow's milk was destroyed by irradiation with a mercury-vapour lamp. Newly-born male piglets were removed from the sow at

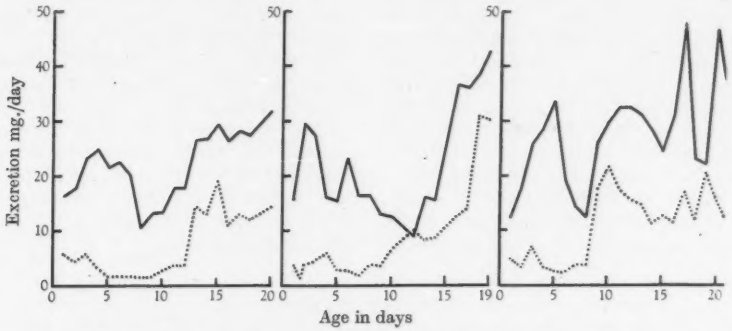

Fig. 1. Total urinary excretion of ascorbic acid (mg./day) by piglets receiving (——) or not receiving (.....) this factor.

birth, kept separate in metabolism cages, and given successively these diets. Litter-mates received the same diets to which ascorbic acid had been added to make its concentration equivalent to that in sow's colostrum and milk. During the first 24 hr. the piglets were fed by hand at 2 hr. intervals; subsequently they helped themselves from self-feeders which were refilled four times during the day. The piglets on both diets grew satisfactorily. There was some indication that the control piglets utilized their food more efficiently than those on the deficient diet.

The urine of each piglet was collected at intervals and kept at 0°. Samples from 24 hr. collections were analyzed each day for ascorbic acid, using a modification of Stewart & Sharp's (1945) indophenol method. Tests showed that there was no loss of ascorbic acid during collection and storage.

The results for three pairs of piglets are shown in Fig. 1. The piglets on the vitamin C-free diet excreted small amounts of ascorbic acid during the first 8–10 days of life; a pronounced increase then occurred and the subsequent secretion, although not constant was markedly greater than that during the first period. The control piglets excreted substantial quantities during the first few days when colostrum was fed; the excretion then fell and rose again at about the same time as it increased in the deficient pigs.

This marked change in excretion at 8–10 days would suggest either that ascorbic acid synthesis does not begin until the piglet is about a week old, or that there is then a marked change in the requirement of vitamin C.

The piglets were killed after 3 weeks and samples of various body tissues were analyzed for ascorbic acid. With the exception of the adrenals, where the content was similar for all piglets, the tissues of the deficient animals contained only about half as much vitamin C as those of the controls.

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Metabolism of Phenanthrene. By E. Boyland and G. Wolf

A compound was isolated from the urine of rats injected with phenanthrene which is apparently identical with that reported by Young (1947). It is optically inactive and melts at 189–190°. From its analysis and by analogy with the metabolism of anthracene and naphthalene, it is thought to be a dihydroxydihydrophenanthrene. It gives a diacetate (m.p. 171°) and is easily dehydrated by aqueous mineral acid to a phenanthrol. This, by comparison with a synthetic specimen and through derivatives, was shown to be 9-phenanthrol. The phenanthrenediol is therefore probably 9:10-dihydroxy-9:10-dihydrophenanthrene. On oxidation with potassium permanganate it yields 9:10-phenanthraquinone and with lead tetra-acetate diphenyl-2:2′-dialdehyde. A preliminary investigation of the ultra-violet absorption spectrum of the compound, kindly carried out by Dr Roe and Mr Beale, confirms this structure, as the spectrum resembles that of 9:10-dihydrophenanthrene.

The 9:10-dihydroxy-9:10-dihydrophenanthrene prepared from phenanthrene by osmic acid oxidation according to Criegee, Marchand & Wannowius (1942), was not identical with the phenanthrenediol isolated from rat urine.

From preliminary investigations it appears that rabbits dosed with phenanthrene excrete a mixture of phenanthrene diols in the urine, as not only has 9:10-dihydroxy-9:10-dihydrophenanthrene been isolated, but also a phenanthrene-diol which yields a mixture of 1-phenanthrol and 2-phenanthrol on dehydration in ethanolic hydrochloric acid. This diol is therefore presumably 1:2-dihydroxy-1:2-dihydrophenanthrene.

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The Effect of Lipoid and Non-lipoid Solvents on the Rate of Elimination and the Carcinogenic Potency of 3:4-Benzpyrene after Subcutaneous Injection in Mice. By H. Weil-Malherbe. (*Department of Physiology, Medical School, University of Durham*)

It has been shown (Weil-Malherbe & Dickens, 1946) that the use of synthetic tricaprylin containing low concentrations (3%) of cholesterol or phospholipids as a solvent for the subcutaneous injection of 3:4-benzpyrene may profoundly affect the rate of benzpyrene elimination and the incidence of induced sarcomas. The higher rate of elimination caused by the addition of cholesterol was associated with a higher tumour incidence, whereas in presence of phospholipids a slower elimination was observed together with a lengthening of the latent period of induced sarcomas. No such correlation could be obtained by ourselves or others when more complex natural oils and fats were used as solvents. A systematic study of simple synthetic or near-synthetic solvents was therefore undertaken in order to obtain confirmatory evidence and, if possible, an explanation of the mechanism of the 'solvent effect'.

Mice were subcutaneously injected with tricaprylin solutions of 3:4-benzpyrene containing one or more of a number of additional compounds. The effect of these additions on the rate of benzpyrene elimination was studied. The substances tested mainly belonged to the two classes of antioxidants or sterols.

Ascorbyl palmitate in 2% solution was the only substance with a probable inhibitory effect, while α-tocopherol caused a marked acceleration. Amongst sterols cholesterol, cholestanol and sitosterol increased the elimination rate whereas epi-cholestanol, coprostanol, epi-coprostanol, stigmasterol, 7-dehydrocholesterol and ergosterol were inactive. This is exactly the situation found by Davis, Krahl & Clowes (1940) with regard to the ability of sterols to form association complexes with benzpyrene in surface films.

The higher rate of benzpyrene elimination caused by 3% cholestanol is associated with a higher incidence of induced sarcomas. The delay in benzpyrene elimination in the presence of 2% ascorbyl palmitate is only temporary and lasts for about 6–7 weeks. It is probably followed by a phase of accelerated elimination. Accordingly there was no effect of ascorbyl palmitate on tumour incidence.

The association between increased elimination rate and formation of co-ordination compounds is further strengthened by the fact that after injection of aqueous benzpyrene solutions made up with the aid of solubilizers the elimination is greatly accelerated. This is not due to the different physical conditions since benzpyrene if injected in ethereal solution or in aqueous colloidal solution is eliminated at a rate not much different from that observed with several oily solvents.

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The Metabolism of Nicotinamide and Related Compounds in Various Mammalian Species. By P. Ellinger and M. M. Abdel Kader. (*The Lister Institute, London, S.W.* 1)

A systematic comparative examination was carried out on nicotinamide metabolites eliminated in the urine by various mammals without and after extra dietary intake of nicotinamide (Nam), nicotinic acid (Nac) or nikethamide (Nik). Metabolites claimed to be observed by various workers are Nam, Nac, nicotinamide methochloride (NM), trigonelline (trig.) and nicotinuric acid. Investigations were performed on man, rat (2 strains), cat (young), guinea-pig, and rabbit (kept on cabbage or oats). The dietary intake (μg./day) was: man, not estimated; rat 0·212 Nam, 0·370 Nac; cat 1·025 Nam, 3·450 Nac; guinea-pig 0·879 Nam, 0·315 Nac; rabbit (cabbage) 1·600 Nam, 1·600 Nac; (oats) 0·208 Nam, 0·336 Nac. 24 hr. samples of urine were collected and examined for Nam, Nac, trig. and nicotinuric acid by a modified Bandier & Hold (1939) method and microbiologically by Barton-Wright's (1944) method and NM by a modification of Huff & Perlzweig's (1947) acetone method. Various doses of Nam, Nac or Nik were given to man subcutaneously and to the animals intraperitoneally. The main results are given in Tables 1 and 2.

In guinea-pigs and rabbits without extra dietary intake of Nam derivatives the acetone method showed some positive results, the values of which were not increased by injection of either of the compounds. The positive result was probably caused by urinary elimination of the coenzymes.

Rabbits eliminated after injection of Nik for about 16 hr. a urine which exhibited a strong purplish fluorescence in the 366 mμ mercury line. This

fluorescence was caused by one, or possibly two or three hitherto unknown pigments which have been isolated and the properties of which are under examination.

Man, rat and cat amidate Nac and methylate Nam. Guinea-pig desaminates Nam but does not methylate Nac, rabbit desaminates Nam but methylates Nac. Nik is by all five species metabolized to Nam by removing the ethyl groups from the amino-N; this confirms the conclusions of Ellinger, Fraenkel & Abdel Kader (1947).

Table 1. *Spontaneous urinary elimination of metabolites*

(μg./day; mean of 8–53 experiments)

Species	Metabolite eliminated		
	Nam	Nac	NM
Man	2000 [635–4080]	810 [250–1260]	8630 [3210–12580]
Rat (albino)	75·5 [4–116]	0	103 [31–210]
Rat (hooded)	139·2 [84–165]	0	955 [450–2010]
Cat	0	0	288 [66–403]
Guinea-pig	0	0	0
Rabbit (on cabbage)	0	878 [503–1200]	0
Rabbit (on oats)	0	1187 [7010–1544]	0

Neither trigonelline nor nicotinuric acid were eliminated. Numbers in brackets are limiting values.

Table 2. *Urinary elimination of metabolites after injection of Nam, Nac, or Nik*

(% of injected; mean of 2–8 experiments)

Species	Compound injected (μg.)	Metabolite eliminated			
		Nam	Nac	NM	Trig.
Man	Nam 100	1·82	0	25·0	0
	Nac 100	2·48	0·89	21·2	0
	Nik 100	6·30	0	15·6	0
Rat (albino)	Nam 20	23·7	0	9·3	0
	Nac 20	23·8	27·0	1·8	0
	Nik 20	36·8	0	10·0	0
Rat (hooded)	Nam 20	15·0	0	29·7	0
	Nac 20	24·1	27·7	15·8	0
	Nik 20	36·8	0	33·1	0
Cat	Nam 40	34·8	0	28·5	0
	Nac 40	12·8	9·8	24·2	0
	Nik 40	18·9	0	16·1	0
Guinea-pig	Nam 200	0	24·2	0	0
	Nac 200	0	82·2	0	0
	Nik 50	19·2	40·8	0	0
Rabbit (on cabbage)	Nam 200	11·8	14·4	0	9·2
	Nac 200	0	63·4	0	33·5
	Nik 200	4·8	9·9	0	6·9
Rabbit (on oats)	Nam 200	7·5	13·0	0	6·4
	Nac 200	0	50·0	0	29·4

No nicotinuric acid was eliminated.

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A Colorimetric Method for the Estimation of Ergothioneine in Human Blood. By A. L. LATNER. (*Department of Chemical Pathology, King's College, University of Durham*)

The methods available for the estimation of ergothioneine in blood are those of Hunter (1928) and Behre & Benedict (1929). The latter method, depending as it does on a molybdic acid reduction, would not appear to be sufficiently specific.

It has been shown that the colour reaction involved in the former method, although excellent for aqueous solutions, gives poor results with human blood. Three specimens of blood examined by this technique gave zero values and no recovery of added ergothioneine. The reaction seems to be adversely affected by the presence of free uric acid.

A new method has been elaborated, which depends on the fact that ergothionene after hydrolysis with 10*N* NaOH will give a red colour when treated with $NaNO_2$ and sulphanilic acid under controlled conditions. Good recoveries are obtained both with solutions of ergothioneine HCl and ultrafiltrates of plasma and whole blood previously diluted 1 : 4 with distilled water.

The applicability of the method to molybdic acid filtrates is under investigation, as it is felt that the elimination of ultrafiltration would make the technique more suitable for routine clinical purposes.

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Blood Ergothioneine Values in Normal Individuals and Thyrotoxicosis. By A. L. LATNER and R. MOWBRAY. (*Departments of Chemical Pathology and Medicine, King's College, University of Durham*)

Since it was demonstrated that ergothioneine acts as a thyroid inhibitor (Lawson & Rimington, 1947), much interest has been aroused with regard to the possible significance of this substance in thyrotoxicosis, as a factor in both aetiology and treatment.

It became essential to define the normal range of ergothioneine values in human blood in order to demonstrate any changes that might occur in thyrotoxicosis. Unfortunately, the methods of estimation then available were not found suitable for this purpose.

Using the method described by one of us in the previous communication, it has been shown that the fasting red blood cell ergothioneine levels were lower than normal in four cases of thyrotoxicosis and low normal in two others. Further cases are being examined. The ergothioneine content of normal and abnormal human thyroid glands is also under investigation.

Ergothioneine is apparently not combined with plasma proteins when added to blood and so could be easily and rapidly excreted by the human kidney after intravenous injection. The thiouracils on the other hand are known to combine with plasma protein and are therefore protected from excretion. These facts might well explain the recent failure of Astwood & Stanley (1947) to demonstrate thyroid inhibitor activity of ergothioneine injected intravenously.

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The Chemical Nature of Aerosporin. By TUDOR S. G. JONES. (*The Wellcome Chemical Research Laboratories, Langley Court, Beckenham, Kent*)

An antibiotic, aerosporin, was first described by Ainsworth, Brown & Brownlee (1947) as the product of the culture on a simple medium of a strain of *B. aerosporus* Greer. It was reported to be a basic substance, forming salts with mineral acids. It and the product of its hydrolysis with acids have since been found to give the ninhydrin reaction and to be optically active (Catch, 1947). The results of partition chromatography on paper (Consden, Gordon & Martin, 1944) suggest that the most active preparations, which when isolated by a variety of preparative methods attain an almost constant level of activity, are still not homogeneous. The intact material behaves as a basic polypeptide and exhibits the usual behaviour when chromatographed on paper, i.e. in butanol-acetic acid mixture, collidine or neutral phenol, it moves slowly and in ammoniacal phenol it moves almost as quickly as the solvent front. Electrometric titration shows two regions for the acceptance of protons having pK values of 10·5 and 5·8. Keeping in alkaline solution (pH 12, 4 hr.) alters the titration curve; there now appear three regions of pK 10·5, 7·3 and 2·35.

A number of preparations, after acid hydrolysis, have been found consistently to give three spots on the paper chromatogram, using a number of

solvents. Two of these spots correspond with leucine and threonine. A single third spot of characteristic shape and colour indicates the presence of basic material, reacting with ninhydrin, and of low R_F values in neutral solvents and of high R_F values in alkaline media. Preparative chromatograms, cut to separate the two portions of the spot, and eluted with water, disproved the apparent inhomogeneity. The following amino-acids have been found to differ from this basic material: lysine, ornithine, homolysine, histidine, arginine, citrulline, β-alanine and glutamic acid.

The quantitative determination, in acid hydrolysates, of leucine and threonine on the paper chromatogram by the method of Woiwod (1948), indicated a ratio of 1 : 3.

The culture medium, after growth of the organism, and at various stages in the isolation of aerosporin, was found to contain *c.* 1 mg./ml. of alanine. This was absent from the original medium and no other amino-acid or peptide appeared to be present in this concentration either before or after growth of the organism. The amino-acid was separated from the culture medium by the use of the ion-exchange resin Zeokarb 215 (Permutit Co. Ltd.) by the method of Bendall, Partridge & Westall (1947) and was found to resist acid hydrolysis, consistent with its simple amino-acid nature.

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Colorimetric Estimation of Manganese by Means of the Folin-Ciocalteu Phenol Reagent. By M. A. M. Abul-Fadl

DEMONSTRATIONS

A Photoelectric Flame Photometer. By W. R. Domingo, W. Klyne and W. Weedon. (*Postgraduate Medical School of London, W.* 12)

The apparatus resembles in principle that of Barnes, Richardson, Berry & Hood (1945), and can be used for the determination of sodium and potassium in biological fluids. The solution, whose Na or K content is to be determined, is introduced as a fine spray into a controlled air-acetylene flame. The intensity of the emitted light (589 and 596 mμ lines for Na; 766 and 770 mμ lines for K) is measured by the response of a photoelectric cell placed behind suitable filters, and serves to determine the concentration of Na or K in the solution. The instrument is calibrated by a series of standard Na or K solutions, and must be repeatedly checked with these during use.

The following dilutions are convenient: plasma for Na, 1 in 100; serum for K, 1 in 10; urine for Na, 1 in 200; urine for K, 1 in 100.

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The Determination of Small Quantities of Water in Acetone. By W. Klyne. (*Postgraduate Medical School of London, W.* 12)

Small quantities of water in acetone can be determined by measuring the temperature at which the acetone gives a homogeneous mixture with di*cyclo*hexyl ($C_6H_{11}.C_6H_{11}$)—cf. Robertson (1943), determination of water in ethanol; Rising & Hicks (1926), determination of water in methanol.

The sample of acetone (1·0 ml.) and di*cyclo*hexyl (2·0 ml.) are mixed and warmed slowly; the temperature at which the cloudy mixture becomes homogeneous (clearing temperature C.T.) is noted.

% water (v/v) in acetone	C.T.	% water (v/v) in acetone	C.T.
0·0	16·0°	0·6	27·0°
0·1	18·2°	0·8	30·7°
0·2	20·1°	1·0	34·2°
0·3	21·9°	1·2	37·7°
0·4	23·6°	1·5	43·1°
0·5	25·3°	2·0	52·0°

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Bilirubin: Modified Procedure without Precipitation of Proteins. By R. V. Coxon and E. J. King

Colorimetric Estimation of Manganese by means of the Folin-Ciocalteu Phenol Reagent. By M. A. M. Abul-Fadl. (*Postgraduate Medical School of London, W.* 12)

Methods for determining Mn involve its oxidation to the heptavalent state and measurement of the resulting pink colour. Other colour reactions have been developed based on the catalytic properties of Mn (e.g. Szebelledy & Bartfai, 1936; Wiese & Johnson, 1939; Kun, 1947).

The method described here is an application of the reducing properties of $MnCO_3$ on the phosphotungstic-phosphomolybdic phenol reagent of Folin & Ciocalteu (1927). Conditions under which strict correlation exists between the extent of the reduction and the concentration of the Mn^{++} have been determined. Certain amino-acids (e.g. glycine, alanine) were found not only to improve the final blue colour and to eliminate any turbidity due to the insolubility of the $MnCO_3$, but also to increase considerably the reduction of phenol reagent by $MnCO_3$.

A 2 ml. sample containing 15–60 μg. Mn^{++} is treated with 1 ml. 7·5% (w/v) glycine. To this mixture 1 ml. dilute (1 in 3) Folin & Ciocalteu reagent is added, followed by 1 ml. 25% (w/v) Na_2CO_3 solution. The mixture is shaken and allowed to stand for 15–20 min. at room temperature for the maximum colour to develop. Suitable standards and blanks are prepared at the same time.

Al, Ba, Ca, Cr, Cu^{++}, Mg, Fe^{+++}, Sn^{+++}, Sr and Zn were without effect except in amounts sufficient to precipitate carbonate when Na_2CO_3 was added (any precipitate can be spun down without affecting the blue colour). Fe^{++}, Sn^{++}, and Cu^{+}, produce an intense blue colour with the phenol reagent. This can be easily overcome by boiling the solution with a few drops of conc. HNO_3, when the metals will thus be oxidized without affecting the Mn^{++}. Co, and to a lesser extent Ni, interferes by producing a similar blue colour, in presence of glycine, but not in its absence.

Recoveries of nearly 100% have been obtained from urines to which 12–60 μg. Mn^{++} have been added after evaporating the urine to dryness and extracting the previously ignited residue with acid. This treatment seems not to affect the Mn^{++}, since the blue-colour reaction with phenol reagent proceeds normally.

The method is somewhat more sensitive than the standard permanganate method. Its advantages lie in its simplicity, reproducibility, and in the very stable and easily measurable blue colours formed. It eliminates the difficulties of oxidation and of colour variation involved in the permanganate methods. It can be adapted for water analysis as well as for biological purposes.

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Determination of Silica and Alumina in Coal Miners' Lungs. By Inge Gernow. (Introduced by E. J. King)

An Improved Technique for Blood Iron Estimations with Undiluted Titanous Chloride from a Micrometer Burette made from a Tuberculin Syringe. By I. D. P. Wootton and E. J. King. (*Postgraduate Medical School of London, Ducane Rd., W.* 12)

This modification of the popular titanous chloride method for blood iron estimations lends itself to considerable accuracy with the simplest manipulations. Blood (5 ml.) is washed with distilled water from a pipette calibrated to contain into a 50 ml. pyrex beaker, a few drops of conc. HNO_3 are added and the beaker is capped with filter paper. The mixture is cautiously evaporated to dryness and then heated more strongly until fuming ceases. The beaker is placed overnight in a muffle furnace at 400°, the residue dissolved by warming with 5 ml. conc. HCl, and the solution diluted to 20 ml. with water. The indicator (1 ml. of 20% KCNS) is added and the contents titrated with undiluted commercial $TiCl_3$ to a colourless end-point.

The $TiCl_3$ is contained in a 'micrometer syringe burette', which consists of a horizontally mounted all-glass 1 ml. syringe, the plunger being driven by

a micrometer screw (Trevan, 1925); the smallest division corresponds to a delivery of 0·0002 ml. The fused-on glass capillary outlet has a turned-up tip to minimize diffusion. It dips into the iron solution together with a tube delivering CO_2 for continuous stirring. The $TiCl_3$ in the burette, which is sufficient for 6–8 titrations, does not deteriorate over a period of an hour, and titrations can be made slowly to a stable end-point.

Titrations of the blood solutions are alternated with titrations of a standard iron solution washed from the same pipette to avoid calibration errors. The stock iron standard is an acid solution of ferric ammonium sulphate containing approx. 5 mg. Fe/ml.; it is standardized by iodimetric titration against $Na_2S_2O_3$ (Treadwell & Hall, 1930). The working iron standard is a 1 : 10 dilution of this, standardized by $TiCl_3$ titration against a solution of 99·7% pure iron wire. The two methods of standardization have agreed within 0·15%.

The precision of the titration procedure can be judged from a simple test. A series of eight equal volumes of a standard iron solution were titrated with a single burette-full of $TiCl_3$. The relative difference between the greatest and least titration figures was less than 0·2%.

It has been found that the glass parts of the apparatus may be replaced without loss of accuracy by a tuberculin syringe with a fused-on capillary outlet.

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Experiments with Mineral Dusts in the Lungs of Animals. By E. J. King, C. V. Harrison and S. C. Ray

Differential Determination of Reduced and Oxidized Haemoglobin with a Photoelectric Photometer. By G. Bull and K. W. Lovel. (Introduced by E. J. King)

An Aid to Paper Partition Chromatography. By D. M. P. Phillips. (*Courtauld Institute of Biochemistry, Middlesex Hospital, W.* 1)

Two devices are demonstrated which allow the rapid measurement of the movement of a spot or band relative to the solvent front (R_F values) on paper partition chromatograms, by direct measurement. Both can be made very cheaply and quickly and have the advantage that any one component of a mixture can just as easily be chosen as standard and the other components referred to this instead of the usual solvent front standard of $R_F = 1$.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **264th Meeting** *of the Biochemical Society was held at the Westminster Hospital Medical School, 17 Horseferry Road, London, S.W. 1, on Friday, 13 February 1948, at 2 p.m., when the following papers were read:*

COMMUNICATIONS

Studies on the Liver Catalase of Normal and Cancerous Rats. By H. WEIL-MALHERBE and R. SCHADE. (*Departments of Physiology and Pathology, Medical School, University of Durham, Newcastle-upon-Tyne*)

The reduction of catalase concentration in the liver of animals bearing a rapidly growing tumour elsewhere in the body has been extensively studied by Greenstein and his group (1941–3), but the mechanism of its causation is still obscure. There are two obvious possibilities: either a toxic principle which inhibits catalase itself or its synthesis is elaborated by the tumour or the tumour causes the withdrawal of a component essential for the synthesis or protection of liver catalase. Since Greenstein found no indication for the presence of a catalase inhibitor, various other hypotheses were tested. In view of the extensive degradation processes accompanying uncontrolled invasive growth and the increased quantities of proteoses and polypeptides present in the blood (Winzler & Burk, 1944) the effect of parenteral injection of foreign protein or protein split products on the liver catalase of normal rats was investigated. On the other hand, the exaggerated demands of a growing tumour for nitrogenous building materials suggested an inhibition of catalase synthesis owing to lack of supplies of required amino-acids. Consequently the effect on liver catalase of keeping tumour-bearing rats on diets of different protein levels was studied.

A high-protein diet (45 % casein) caused a significantly faster growth of the tumours and a smaller loss of body weight in tumour-bearing rats than a low-protein diet (8 % casein). The protein content of the liver was slightly reduced in normal rats on the low-protein diet. It was considerably lowered in all tumour-bearing rats, irrespective of the protein level of the diet.

The catalase concentration in *normal* rat liver extracts of equal protein content was not significantly changed either by different dietary protein levels or by daily intraperitoneal injections of a 10 % solution of peptone. It was however significantly raised by daily intraperitoneal injections of sheep serum.

The growth of transplanted Jensen rat sarcomas led to a fall of liver catalase concentration which in extreme cases reached 5 % of normal. The decrease of catalase concentration with the ratio of body weight/tumour weight followed an exponential course, although the individual scatter was very pronounced even in similar stages of tumour development. The protein level of the diet had no significant effect on the curve, not even in the initial stages. Serum injections in tumour-bearing rats were likewise ineffective when they were started 11 days after transplantation. When started 4 or 5 days after transplantation they were followed by regression of the tumour.

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The Constitution of Mycophenolic Acid. By J. H. BIRKINSHAW, A. BRACKEN and E. N. MORGAN. (*Department of Biochemistry, London School of Hygiene and Tropical Medicine, London, W.C. 1*)

Clutterbuck & Raistrick (1933) in the light of considerable experimental work, for details of which the original paper should be consulted, put forward a partial structural formula for mycophenolic acid. Further work has led to a revision and amplification of this formula.

When mycophenolic acid and some of its derivatives are subjected to ozonization a smooth cleavage takes place at the double bond into an aliphatic and a benzenoid residue. Thus mycophenolic acid methyl ether $C_{18}H_{22}O_6$, containing two OMe groups, is split into levulic acid and an

aldehyde $C_{13}H_{14}O_5$, retaining the two OMe groups. The carboxy group known to be present in the original molecule must therefore be attached to the side chain and not to the benzene nucleus.

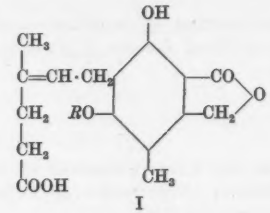
The aldehyde $C_{13}H_{14}O_5$ is converted by hypoiodite into an acid $C_{13}H_{14}O_6$, as represented by the reaction $\cdot CHO \rightarrow \cdot COOH$. On acetylation the aldehyde affords an enol acetate which undergoes further cleavage by ozone at the new double bond with production of an aldehyde $C_{12}H_{12}O_5$. These reactions indicate that the $\cdot CHO$ group is not directly attached to the benzene ring but is linked through a $\cdot CH_2\cdot$ group.

The acid $C_{13}H_{14}O_6$ can be further oxidized by alkaline $KMnO_4$ to give a tribasic acid isolated as the anhydride $C_{13}H_{12}O_7$, the relevant reaction being

$$—CH_2\cdot O\cdot CO— \rightarrow —CO\cdot O\cdot CO—.$$

The substituents in the ring are therefore: two OMe groups *meta* to each other; $—CH_2\cdot CHO$, $—CH_2\cdot O\cdot CO—$ and $—CH_3$, all six hydrogen atoms of the ring being substituted. There are four possible structures; two can be eliminated as unlikely for various reasons, leaving I or II ($R=H$) as the probable structure for *nor*mycophenolic acid, of which I is preferred.

The position of the single methoxyl group in mycophenolic acid itself is undetermined but from the $FeCl_3$ colour reaction and the dichloroquinone chloroimide reaction, which is positive for *nor*mycophenolic and negative for mycophenolic acid it is



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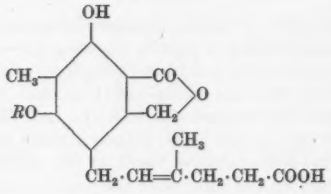

II

likely that the OH is *ortho* to the potential COOH group. Hence mycophenolic acid is represented as I or II ($R=CH_3$), with preference given to I.

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Fuscin, an Antibacterial Pigment from *Oidiodendron fuscum* Robak. By S. E. Michael. (*Department of Biochemistry, London School of Hygiene and Tropical Medicine, London, W.C.* 1)

The mould *Oidiodendron fuscum* Robak when grown on Czapek-Dox medium produces an antibacterial substance. After 3 weeks' incubation at 24° the culture solution has an activity (*Staphylococcus aureus*) of 1 : 40 to 1 : 80. The active substance is readily isolated by aeration of the dark-coloured metabolism solution after filtration from the mycelium. An orange-brown powder separates which is filtered off and which on recrystallization from alcohol is obtained in the form of shining orange plates of m.p. 230°. This substance shows high antibacterial activity and is in fact the main active principle formed by the mould. The average yield is 0·3 g./l. of culture fluid. The name *fuscin* is proposed for this pigment.

In the metabolism solution fuscin is present in the colourless dihydro-form. Dihydrofuscin has actually been isolated from the solution. The oxidation to fuscin appears to be accomplished through the agency of an enzyme.

Fuscin is a compound of quinonoid character, and has the empirical formula $C_{15}H_{16}O_5$, m.p. 230°. It is soluble in organic solvents except petroleum ether, soluble in alkalis with intense purple colour. It is optically inactive; it possesses two C-Me groups, shows one active H in pyridine, none in anisole. Aldehyde reactions are negative. It gives a purplish red $FeCl_3$ reaction in alcohol. It is easily reduced by hydrosulphite or catalytically to the colourless *dihydrofuscin* $C_{15}H_{18}O_5$, m.p. 206°.

Fuscin had an appreciable inhibitory effect on 18 strains of Gram-positive bacteria with limiting dilutions varying from 1 : 80,000 to 1 : 1,280,000. It inhibited 9 Gram-negative strains at limiting dilutions varying from 1 : 5,000 to 1 : 80,000. It showed weak activity against Mycobacteria.

Fuscin reacts with thiol compounds such as cysteine and thioglycollic acid; the colourless crystalline compound formed in the latter case analyzes as fuscin hydrate.1 thioglycollic acid.

This stable substance possesses only slightly lower antibacterial activity than fuscin itself. In the presence of higher concentration of thiol compounds, the activity is in some cases lowered, but not abolished, even with large excess (17 : 1 mol.) of thiol compound. In dilution tests zones of alternating inhibition and full activity were found, similar to the observation of Waksman & Schatz (1945) on streptomycin, and Burkholder (1944) on various antibiotics.

It is concluded that the antibacterial action of fuscin is not due to interaction with SH-groups.

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The Influence of Added Cephalin on the Thymol Turbidity and Flocculation Tests. By N. F. MACLAGAN. (*Department of Chemical Pathology, Westminster Hospital Medical School, London, S.W.* 1)

Much previous work on the mechanism of this test has indicated the essential role of the native serum phospholipids in the production of the turbidity (Maclagan, 1944; Recant, Chargaff & Hanger, 1945; Kunkel & Hoagland, 1947; Maclagan & Bunn, 1947). It therefore appeared desirable to determine whether the addition of phospholipid to the reagent would improve the diagnostic value of the test and to this end sera from 45 miscellaneous cases including 12 of hepatitis in various stages were tested with and without the addition of cephalin. The cephalin was added in ethereal solution (1 % w/v), one drop being added to the 0·05 ml. of serum immediately before addition of the thymol reagent. Observations were made both on the turbidity at 30 min. and flocculation after 18 hr., using the convention recently described for recording flocculation (Maclagan, 1947). The table shows the differences between the results obtained with each test with and without cephalin addition.

Changes in thymol turbidity test produced by addition of cephalin in 45 cases (units)

Positive (12 cases)	No change	Negative (9 cases)
10, 3, 3, 2, 1, 1, 1, 1, 1, 1, 1, 1	24 cases	6, 4, 4, 4, 2, 1, 1, 1, 1

Changes in thymol flocculation test produced by addition of cephalin in 45 cases

Positive (3 cases)	No change	Negative (11 cases)
1, 1, 1	31 cases	4, 3, 2, 2, 2, 1, 1, 1, 1, 1, 1

It will be seen from this that the turbidity may be either increased or decreased by cephalin addition, but is usually unchanged. The changes were rarely such as to change a positive to a negative result or vice versa. With the flocculation test on the other hand the change if any is usually an inhibition of flocculation, which may occasionally be sufficient to reduce a strongly positive to a negative result (e.g. 4+ to 0). No particular type of case appeared to be associated with the presence or absence of this cephalin effect. It would appear therefore that the addition of cephalin is not to be recommended as a routine procedure for serum, although it is of course essential with fractions isolated electrophoretically.

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An Improved Diacetyl Reaction for the Estimation of Urea in Blood. By V. R. WHEATLEY. (*Department of Chemical Pathology, Westminster Hospital Medical School, London, S.W.* 1)

The diacetyl method for blood urea of Barker (1944) has the advantage of being a direct method and in having greater sensitivity than the nesslerization-urease method. It has been found that if the condensation with diacetyl is carried out in the presence of an aromatic amine the colour produced is orange or red instead of yellow. In particular diphenylamine and its derivatives produce intense magenta colorations, that with *N*-phenylanthranilic acid being more than five times as intense as the original

yellow colour with diacetyl. The reaction has been studied in detail and a method for the estimation of blood urea has been devised.

The principle of the method is as follows: the proteins are precipitated with zinc hydroxide, a portion of the filtrate, equivalent to not more than 0·02 ml. blood, is pipetted into a test tube, diacetyl monoxime and phenylanthranilic acid solutions are added followed by 18N sulphuric acid. The tube is placed in a boiling water bath for 10 min., immediately after removal and without cooling, potassium persulphate solution is added, the tube mixed and allowed to stand at room temperature for 15 min. for the colour to develop. It is then read in a suitable colorimeter, using an Ilford spectrum green filter.

The reaction conditions have been investigated. It was found that colour production reached a steady value after 10 min. heating in the water bath. After the addition of persulphate the colour produced reaches a maximum in 15 min., and is stable for several hours. The specificity of the reaction compares favourably with the original diacetyl method except that creatinine gives a similar coloration to urea, but in this case the sensitivity is only one hundredth of that for urea. The method has been compared with the manometric method of Van Slyke & Kugel (1933). Good agreement was obtained with the majority of specimens, 80 % showing difference of less than 3 mg. urea/100 ml; in 10 % of specimens, however, the results differed by 8–10 mg./100 ml. Nevertheless, this method compares favourably with other colorimetric urea methods. It has the disadvantage that the final solution is strongly acid. Attempts to overcome this were unsuccessful since considerable dilution with water causes precipitation of the colour complex, while dilution with alcohol causes the colour to fade.

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The Use of Radioactive Isotopes in Immunological Investigations. 1. Precipitin Reactions with Ovovitellin containing P^{32}. By G. E. Francis and A. Wormall. (*Medical College of St Bartholomew's Hospital, London, E.C.* 1)

P^{32}-containing vitellin and lipovitellin have been separated from eggs laid by hens injected with P^{32}-containing inorganic phosphate, and have been used in precipitin reactions. By the use of these labelled antigens the amount of antigen in the specific lipovitellin-antivitellin precipitates can easily be determined. In other experiments, P^{32}-containing lipid of the eggs has been used to determine the role of the egg phosphatides in the lipovitellin-antivitellin reaction. Evidence was obtained that lipovitellin-antivitellin precipitates contained a much higher ratio of phosphatide P^{32}/protein P^{32} than did the lipovitellin antigens. This preferential carrying down of phosphatide by the precipitate is specific, and it does not occur in precipitin reactions unrelated to vitellin, e.g. in the precipitation of human serum proteins by their antibodies.

The Use of Radioactive Isotopes in Immunological Investigations. 2. Fate of Injected P^{32}-containing Proteins. By T. E. Banks, J. C. Boursnell, Hilary M. Dewey, G. E. Francis, R. Tupper and A. Wormall. (*Medical College of St Bartholomew's Hospital, London, E.C.* 1)

A preliminary study has been made of the fate of the following P^{32}-containing antigens after their intravenous injection into rabbits: phosphorylated human serum proteins, lipovitellin and vitellin. The object of these investigations was to find out which tissues take up the injected antigens, in the hope that useful information might be obtained about the site of antibody formation. The less soluble antigens, lipovitellin (which is soluble in solutions containing 5 % or more NaCl) and vitellin (which is almost insoluble in water or NaCl solutions, and which was used as a fine suspension) were used in the hope that these radioactive compounds would be specifically deposited in the reticulo-endothelial system and certain other tissues of the body.

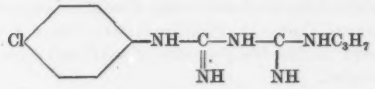
The injected phosphorylated human serum proteins rapidly disappeared from the blood stream, at least three-quarters leaving the blood during the first 3 hr. and well over 90 % during the first 24 hr. after the injection. The disappearance of the injected

lipovitellin was even more rapid; only 20% or less of the injected antigen could be detected in the blood 5–15 min. and not more than 5% 4 or 5 hr. after the injection.

The rabbits receiving intravenous injections of the vitellin suspension died within 1 min. of the injection, presumably not because of the radioactivity of the injected antigen. Appreciable amounts of P^{32}-containing material were found in the lungs and livers of these injected rabbits, with little or none in the heart and spleen.

Some Pharmacological Properties of *p*-Chloroaniline. By L. A. Elson, A. Spinks and Mary M. Tottey

The *p*-chloroaniline molecule is a constituent of the new antimalarial drug Paludrine (I), and is readily

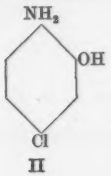


I

formed from the latter by acid hydrolysis (Spinks & Tottey, 1946). It therefore seemed important to investigate the pharmacological properties of *p*-chloroaniline as a potential metabolite of the antimalarial. Its toxicity has been studied in mice and rats. The approximate acute oral LD_{50} in mice is 10 mg./20 g. When administered orally twice daily to mice over 5 days, 2 mg./20 g. resulted in the deaths of approximately 50% of the animals. The main toxic symptoms observed were ataxia and cyanosis. During an extended chronic toxicity test in rats approximately 4 mg./rat/day were administered over a period of 6 months and the animals were then observed until the death of the last rat after 28 months. No pathological change attributable to the compound was found.

A method of estimating the compound was devised and studies were made of the concentrations attained by it in body fluids and tissues of mice, rats and rabbits. In some experiments simultaneous estimations of MHb concentrations were carried out. The compound attains fairly high concentrations of up to 10 mg./100 ml. in the blood and is rather uniformly distributed through the tissues, being localized to a limited extent in fatty tissues. Susceptibility to methaemoglobinaemia decreased in the order: rats; mice; rabbits. 80% conversion of Hb to MHb was observed in rats.

Traces only of free *p*-chloroaniline could be isolated from the urine of rabbits. It appeared to be acylated in mice and rabbits, and in rabbits and rats a pronounced rise in the excretion of ethereal sulphate was detected. 5-Chloro-2-aminophenol (II) was isolated from the urine of rabbits after acid hydrolysis.



II

This metabolite accounted for only a small proportion of the administered *p*-chloroaniline, and other degradative processes must occur. Lack of knowledge of these suggests that the formation of *p*-chloroaniline from Paludrine *in vivo* would be very difficult to detect chemically. However, the high degree of methaemoglobinaemia produced by *p*-chloroaniline in experimental animals is the most pronounced of its pharmacodynamic actions, and forms a sensitive biochemical test for it. No cyanosis has been detected in man or animals after the administration of Paludrine, and it is therefore concluded that free *p*-chloroaniline cannot be formed from the latter in dangerous amount.

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Some Biochemical Properties of Methyl Bromide. By D. R. P. Murray. (*Department of Zoology and Applied Entomology, Imperial College of Science and Technology, London, S.W. 7*)

Methyl bromide is extensively used in U.S.A. as a fumigant and in fire extinguishers. *In vivo* it is generally supposed to hydrolyze to methyl alcohol, which in turn is oxidized to formaldehyde, but this does not adequately explain its high toxicity. The residues of inorganic bromide in foodstuffs after fumigation have likewise been ascribed to hydrolysis, although Laug (1941) and Roehm, Schrader & Stenger (1942) found the extent of these residues varied with the nature of the foodstuff. But these

facts would rather support the view that inorganic bromide arises from a reaction primarily consisting of methylation, viz. $XH + CH_3Br = XCH_3 + HBr$.

In contrast to the long-established use of alkyl halides as 'dry' alkylating reagents in heated sealed tubes, their reactivity in aqueous systems has scarcely been studied. Solutions of various substances were saturated with methyl bromide gas, or filter papers impregnated with the solutions were exposed to the gas at room temperatures. With many nitrogen and sulphur compounds but in no cases with compounds containing only C, H and O (except at extremes of pH permitting hydrolysis), bromide ions were soon detectable. Equilibrium was reached in 24–48 hr. If the filter papers were thoroughly dried no reaction occurred.

The following conditions govern the reactivity of nitrogen compounds. The nitrogen atom must have basic properties (hence amides, uric acid, etc., are inactive), but may be primary, secondary or tertiary, the three types reacting with 3, 2 and 1 molecules of methyl bromide, the latter yielding no free HBr; the final product is thus a quaternary compound. In amino-acids, polypeptides and gelatin disappearance of the amino group has been followed. The basic group must be unionized; salts including zwitterions are inactive. Hence amino-acids require addition of alkali, but peptides and proteins show a slight reactivity at pH 7. Weaker bases, including the important pyridine bases, give full reaction at neutrality or slight acidity. The methylation of nicotinic acid, which proceeds unhampered by presence of starch, etc., may have a bearing on practical fumigation.

N-methylation is apparently the only reaction taking place under these conditions. *O*-Methylation, as described for wool and methyl bromide vapour by Blackburn, Carter & Phillips (1941), has not been observed; in fact the presence of carboxyl groups is immaterial.

Dixon & Needham (1946) have shown that methyl bromide is a vesicant, but these reactions resemble those not of mustard gas, but rather of its sulphone (Boursnell, Francis & Wormall, 1946).

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Flavianates of Lysine. By J. G. Heathcote. (*Department of Biochemistry, King's College, London*)

The first, and only, reference to a salt of lysine with 2:4-dinitro-1-naphthol-7-sulphonic (flavianic) acid is that of Kossel & Gross (1924). These workers claimed the preparation of a flavianate of lysine (m.p. 213°) which had N and S contents corresponding to a mono-derivative. This salt possessed only a very slight solubility in 96% alcohol but was much more soluble in H_2O, dilute mineral acids and flavianic acid solution than the monoflavianates of histidine* and arginine. However, the salt of lysine with flavianic acid, described by Kossel & Gross, has not yet been confirmed. Carl Schmidt (1944) reports briefly that 'flavianates of lysine...have thus far not been isolated'.

By the addition of excess flavianic acid to an aqueous solution of lysine monohydrochloride, a salt has been prepared, corresponding in analysis with the diflavianate of lysine:

Calc. (%): C, 40·32; H, 3·36; N, 10·84; S, 8·28
Found (%): C, 39·97; H, 3·44; N, 10·6; S, 8·59

Lysine diflavianate is precipitated quantitatively (97%) from aqueous solution as pale-yellow platelets which melt, when pure, at *c.* 275° with decomposition. Unless stoichiometric proportions are present, however, a salt with a lower melting point precipitates and recrystallization from dilute flavianic acid solution becomes necessary.

On washing the crystals of diflavianate with ethanol some change of colour occurred towards the orange shade of arginine monoflavianate, together with a lowering of melting point, suggesting the partial formation of a lower salt. Lysine monoflavianate was, in fact, prepared by dissolving the diflavianate in the minimum quantity of hot water and adding excess ethanol to the solution. The orange needles separating from the mixture at 5° melted at 213° with decomposition and had the following analysis:

Calc. (%): C, 41·74; H, 4·35; N, 12·17; S, 6·97
Found (%): C, 40·83; H, 4·58; N, 11·61; S, 6·89

It is evident, therefore, that the salts of lysine which have been prepared are, respectively, the

* Kossel's monoflavianate of histidine was later shown by Vickery (1927) to be contaminated with the diflavianate.

diflavianate, hitherto undescribed, and the monoflavianate, identical with that described by Kossel & Gross.

Excess flavianic acid has been used by some workers to free diets from arginine for nutritional work. Bunney & Rose (1928) found that all histidine was removed at the same time from a casein hydrolysate, by this procedure. It is now apparent that removal of lysine, in part at least, may also take place. The estimation of arginine by precipitation as diflavianate (Vickery, 1940) should also be reconsidered in the light of the present findings. The existence of lysine diflavianate might also explain the previous isolation of several unidentified flavianates from protein hydrolysates, e.g. by Beveridge & Lucas (1944).

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DEMONSTRATIONS

A Simplified Apparatus for the Determination of Oxygen Consumption in Small Animals. By N. F. Maclagan

Estimation of Urinary Pregnanediol. By N. F. Maclagan and V. R. Wheatley

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University College, Gower Street, London, W.C. 1, on Friday, 12 March, 1948, at 2 p.m., when the following papers were read:*

COMMUNICATIONS

The Prosthetic Groups of the Intracellular Haems of *Corynebacterium diphtheriae* and their Relationship to the Cytochromes. By W. A. Rawlinson and J. H. Hale. (*Departments of Chemical Pathology and Bacteriology, University College Hospital Medical School, London*)

The growth and metabolism of toxigenic strains of *Corynebacterium diphtheriae* are influenced by the concentration of inorganic iron salts in the culture fluid. At very low Fe levels growth is poor. The production of coproporphyrin and of toxin is maximal at 0·14 mg. Fe/ml. (Pappenheimer, 1947) and for each additional 4 atoms of Fe, 4 molecules of porphyrin and 1 of toxin fail to appear in the culture fluid but there is an increase of haem components within the cells. Pappenheimer (1947), after identifying cytochrome *b*, suggested that porphyrin and exotoxin were derived from this respiratory carrier. This hypothesis was again offered without further support in the recent paper by Pappenheimer & Hendee (1947).

We have found absorption bands in cell suspensions, reduced with sodium hyposulphite at (601), 562 and 531 mμ. and the addition of pyridine shifts the band maxima to 587, 557 and 527 mμ. These results together with other evidence confirm the findings of Fujita & Kodama (1934) and Pappenheimer & Hendee (1947), that the main haem compounds are cytochrome *b* and cytochrome oxidase.

The intracellular haems from cells grown at relatively high Fe levels have been extracted with acetone-HCl and a crude haem residue obtained free from other pigments. The development of a new process of separation, involving the phase distribution of haems between HCl-pyridine and ether, has facilitated the study of these substances. From the HCl-pyridine phase the prosthetic group of cytochrome *b* has been isolated in crystalline state and identified as protohaemin IX. Determinations have been made of extinction coefficients (as pyridine haemochromogen) of the red-green haem remaining in the ether phase, thereby providing data for the determination of the prosthetic group of cytochrome oxidase in tissue extracts.

The phase separation of haems has been applied to ox heart muscle and the pigment isolated from the ether fraction is similar to that obtained from *C. diphtheriae*. The removal of iron from these green haemins gave a rhodo type porphyrin and further work is in progress to identify its precise structure.

A positive cysteine reaction and oxime formation have indicated the presence of a labile formyl group. Marked changes in absorption spectra are observed after chemical alteration of the labile formyl group in these substances. This fact makes it pertinent to suggest that some of the confusion existing over the cytochrome *a* components, cytochrome oxidase and the respiratory ferment of Warburg might be related rather to the lability of a formyl group than to inherent differences between the protein moieties.

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The Respiratory Quotient of the Mammary Gland. By S. J. Folley and T. H. French. (*National Institute for Research in Dairying, University of Reading*)

A number of authors have reported that the mean R.Q. of the lactating gland, determined by the method of arterio-venous sampling on the cow and goat, is greater than unity. However, apart from the uncertainties inherent in this method, the published values are not altogether decisive because of their variability, many individual results being less than 1. Some reports of experiments on perfused udders also give quotients less than 1 (for references see Kay (1947) and Folley (1948)). A series of determinations on the intact lactating cow made in this laboratory with particular care as to technique of

blood sampling was reasonably consistent and gave a mean quotient of 1·25 (Bottomley & Folley, unpublished experiments).

We have now measured the R.Q. on surviving slices of rat mammary tissue by the method of Dickens & Simer (1931), with bicarbonate-Ringer containing 0·2% glucose as substrate. A total of 26 determinations on 10 lactating rats have given a mean quotient of 1·56, range 1·30–1·86. In the absence of added substrate on the other hand, the R.Q. is below unity. Both in presence and in absence of glucose the oxygen uptake (measured in phosphate buffer by the direct method) is practically linear for 3 hr.; the Q_{O_2} (glucose) of some specimens being equal to − 10.

It appears that lactose is ineffective as a substrate and that the remaining carbohydrate reserves of the tissue are very low; numerous analyses of the glycogen in fresh tissue put its concentration as low as 0·02% in the case of the lactating rat and only slightly more in several other species.

The existence of a high R.Q. together with a fairly active respiration *in vitro*, raises interesting questions in relation to the pathways of glucose metabolism in this tissue.

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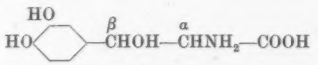
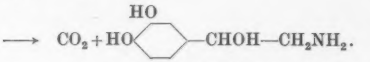
Enzymic Formation of (−)-nor-Adrenaline. By H. Blaschko, Pamela Holton and G. H. Sloane Stanley. (*Department of Pharmacology, University of Oxford*)

β-3:4-Dihydroxyphenylserine, first prepared by Rosenmund & Dornsaft (1919), was tested as substrate of mammalian decarboxylases. It was found that extracts of the guinea-pig's kidney and suprarenal gland did not decarboxylate this amino-acid. The kidney extracts will rapidly decarboxylate L-3:4-dihydroxyphenylalanine, whereas the suprarenal extracts were found to be without dopa decarboxylase activity.

Carbon dioxide was formed when 3:4-dihydroxyphenylserine was incubated anaerobically with an acetone-dried preparation of *Streptococcus faecalis* R which contained L-tyrosine decarboxylase. The amount of CO_2 formed was equivalent to approximately one-half of the amino-acid added. This suggested that the following reaction had taken place:



3:4-dihydroxyphenylserine



nor-adrenaline

We therefore examined the action of the incubated sample on the arterial blood pressure of the spinal cat; a high pressor activity was found. Calculation, from the amount of carbon dioxide formed, of the amine content of our solution and comparison of its pharmacological activity with that of (±)-nor-adrenaline showed that the amine formed had approximately twice the pressor activity of racemic nor-adrenaline; it must therefore have been (−)-nor-adrenaline.

Our findings make it possible to relate the configuration of 3:4-dihydroxyphenylserine to that of (−)-nor-adrenaline. The amino-acid has two asymmetric carbon atoms; four different configurations are therefore theoretically possible. We used a sample of the amino-acid prepared by Dalgliesh & Mann (1947). Dr Mann informs us that this specimen is a racemic mixture, containing only two stereoisomers. Since it is known that the bacterial enzyme is specific for L amino-acids, it follows that one-half of our specimen has the L configuration at the alpha carbon atom and the configuration of (−)-nor-adrenaline at the beta carbon atom.

The authors are grateful to Dr F. G. Mann for the sample of the amino-acid used in these experiments.

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The Amino-acids Present in Trypsin and the Trypsin Inhibitor from Soya Bean. By ELIZABETH WORK. (*Department of Chemical Pathology, University College Hospital Medical School, London*)

Crystalline samples of trypsin and soya bean trypsin inhibitor were hydrolyzed with 6N-HCl and subjected to paper partition chromatography according to the method of Consden, Gordon & Martin (1944). The following amino-acids were identified in both substances: aspartic acid, glutamic acid, glycine, alanine, serine, threonine, valine, leucine, isoleucine, histidine, proline, tyrosine, arginine, lysine, cystine. Besides these, the inhibitor contained normal amounts of phenylalanine and methionine. The amount of these two amino-acids present in trypsin was exceptionally low, probably less than 0·5%.

Leucine and isoleucine were easily separated and identified by a one-dimensional technique using a *tert*-amyl alcohol/water mixture in an atmosphere of diethylamine, the solvent being allowed to run over the paper for 4 days. By this method, a good separation of phenylalanine and methionine was also obtained.

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The Absorption of Vitamin A. By EVA EDEN (*Dunn Nutritional Laboratory*) and K. C. SELLERS (*Institute of Animal Pathology, University of Cambridge*). (Introduced by T. MOORE)

Drummond, Bell & Palmer (1935), and McCoord, Breese & Baum (1943) demonstrated an increased concentration of vitamin A in the lymph collected from the thoracic duct following oral administration of the vitamin. Popper & Volk (1944) observed a fluorescence in the lacteals of the rat's gut, typical of vitamin A 25 min. after similar treatment. In the present investigation, the portal blood as well as the lymph was studied as a possible pathway of absorption of vitamin A.

Eleven yearling bullocks, weighing 150–250 kg., were dosed orally with vitamin A in the form of halibut-liver oil, emulsified in reconstituted separated milk. The dose of vitamin A was adjusted to 5000 i.u./kg. of body weight, and the animals were slaughtered at intervals varying from 2 to 24 hr. after dosing. Two undosed animals were included in the series. Vitamin A was estimated in the jugular blood before dosing and in the systemic and portal bloods collected at slaughter. At the same time lymph glands from the intestinal and other regions of the body were removed, allowed to drain, and vitamin A estimated in the lymph thus obtained.

The vitamin A content of the blood increased after dosing, but in no case was the level in the portal blood greater than that of the systemic blood. Vitamin A levels in the lymph draining the mesenteric glands, more especially those draining the duodenum, rose significantly, but not in the lymph collected from other regions of the body.

	Vitamin A (i.u./100 ml.)	
	Undosed	Dosed
Blood: Before dosing	87	72
At slaughter: Systemic	83	162
Portal	90	147
Lymph: Intestinal lymph:		
Duodenal	225	1500
Jejunal	185	1028
Ileal	130	459
Colonic	132	169
Non-intestinal lymph	130	175

Confirmatory results have been obtained in experiments performed with sheep and rats. A few samples of intestinal lymphs from sheep have been fractionated, and in these about 95% of the vitamin A was in the ester form but small amounts of vitamin alcohol were detected.

So far we have been unable to obtain conclusive evidence that vitamin A is carried from the intestine by the portal blood. On the other hand the lymph draining the small intestine appears to be the main pathway by which the vitamin A reaches the general circulation.

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Inhibition of Rat-Liver Succinoxidase by Synthetic Oestrogens and Related Substances. By E. M. CASE and F. DICKENS. (*Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W.* 1)

It was shown by McShan & Meyer (1946) that some oestrogens, particularly synthetic oestrogens of the stilboestrol series, were powerful inhibitors of the succinoxidase system of rat liver and pituitary tissues in concentrations of the order of 10^{-4}M. Some evidence was produced that certain structural modifications led to parallel diminution in both oestrogenic and inhibitory potency. If such a consistent and specific relation could be established it would have obvious importance as a potential clue to the physiological mechanism of oestrogenic action *in vivo* and might moreover throw light on the not unconnected phenomenon of carcinogenicity. However, after examination of an extended series of oestrogenic substances and compounds closely allied chemically we can find no grounds for such a generalization. Some non-oestrogenic substances inhibit strongly, while certain highly potent oestrogens, e.g. 7-methyl*bis*dehydrodoisynolic acid and β-(6-methoxy-2-naphthyl)-α, α-dimethyl-*n*-valeric acid are almost completely devoid of influence upon rat-liver succinoxidase. It is likely that the presence of phenolic hydroxyl groups in the molecule is associated with the inhibition.

McShan & Meyer considered that the inhibition which they observed was exerted upon the cytochrome oxidase component of the system and not upon succinic dehydrogenase. We found that this was by no means invariably the case for all the compounds we tested. One component or the other, or both, might be affected, in unpredictable relative degrees. However, one substance which was tried, viz. *p*-*p*′-dihydroxystilbene, yielded results of peculiar interest in that a concentration (2×10^{-4}M) which totally inhibited the overall oxidation of succinate was without effect upon either cytochrome oxidase or succinic dehydrogenase. The interference must therefore occur at some stage or process which links these two enzyme complexes. The existence of such an intermediate stage has been postulated on a number of occasions, from the observations of Hopkins, Lutwak-Mann & Morgan (1939) to those of Stoppani (1947) and of Ball, Anfinsen & Cooper (1947). It should be possible to employ this specific inhibitor to make some progress towards its characterization.

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Studies on the Synthesis of Ethereal Sulphates *in vivo*. By J. C. LAIDLAW and L. YOUNG. (*Department of Biochemistry, University of Toronto*)

There has been some controversy concerning the possible utilization of administered inorganic sulphate for the synthesis of ethereal sulphates in the animal body (Rhode, 1923; Muldoon, Shiple & Sherwin, 1924; Hele, 1924). This matter has now been studied in the rat with the aid of Na_2SO_4 labelled with radioactive sulphur (S^{35}). In this work 2-naphthylamine and 2-naphthol were employed as ethereal sulphate precursors, for in earlier work in this laboratory it had been established by the isolation of the ethereal sulphates from the urine of the rat that 2-naphthylamine is converted in this animal to the ethereal sulphate of 2-amino-1-naphthol (Manson, 1947) and that 2-naphthol is converted to 2-naphthylsulphuric acid (Berenbom, 1947).

When radioactive Na_2SO_4 was given to rats by intraperitoneal injection, 5–6 % of the administered S^{35} was excreted in the urine in the following 72 hr. in the ethereal sulphate fraction and 86–90 % in the inorganic sulphate fraction. When these rats were dosed with 2-naphthylamine by stomach tube or by subcutaneous injection and at the same time were injected intraperitoneally with radioactive Na_2SO_4, it was found that in the following 72 hr. 44–48 % of the administered S^{35} was excreted in the ethereal sulphate fraction of the urine and 44–49 % in the inorganic sulphate fraction. The amounts of Na_2SO_4 injected (approx. 0·1 mg. of sulphate S per rat) were small and less than 2 % of the average daily excretion of total sulphate S in the urine. The results obtained suggested that the administered sulphate had parti-

cipated in the formation of the ethereal sulphate of 2-amino-1-naphthol in the rat. This was confirmed by the isolation of the radioactive ethereal sulphate of 2-amino-1-naphthol from the urine of rats dosed by subcutaneous injection with 2-naphthylamine and injected intraperitoneally with radioactive Na_2SO_4. Furthermore, radioactive 2-naphthylsulphuric acid was isolated from the urine of rats which had been given 2-naphthol by subcutaneous injection and radioactive Na_2SO_4 by intraperitoneal injection. The specific activities of the isolated radioactive ethereal sulphates did not change significantly on repeated crystallization of the compounds. When radioactive Na_2SO_4 was added to rat urine containing the ethereal sulphate of 2-amino-1-naphthol or 2-naphthol no evidence of a transfer of S^{35} to the ethereal sulphate fraction of the urine in the following 72 hr. was obtained.

From the results of the present work it can be concluded that administered inorganic sulphate is utilized in the formation of ethereal sulphates in the rat.

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The Influence of Thyroxine and 2:4-Dinitrophenol on the Succinoxidase Activity of Rat Liver.

By H. G. WILLIAMS-ASHMAN. (*Department of Biochemistry, University College, London, W.C.* 1)

It has been reported that the succinoxidase activity of rat liver is increased on the administration of thyroid powder (Tipton, Leath, Tipton & Nixon, 1945) and also of thyroxine and thyrotropic hormone (Tipton & Nixon, 1946). The succinoxidase activity of rat liver is diminished in riboflavin deficiency and can be restored to normal levels on the subsequent administration of riboflavin (Axelrod, Swingle & Elvehjem, 1942); it has also been observed that the simultaneous administration of thiamine and vitamin B_2 complex will prevent the loss of weight which normally accompanies the administration of thyroid hormone to rats (Drill & Overman, 1941).

Thyroxine was found to increase the liver succinoxidase activity of adult male rats *in vivo*. The experiments were performed with paired feeding controls, the diet being fortified with thiamine, riboflavin, nicotinamide, pyridoxine and pantothenate. Administration of thyroxine over a period of 8 days resulted in a mean loss of weight in the experimental animals of less than 2%; during the same period the controls suffered a mean gain in weight of less than 2%.

The administration of large doses of 2:4-dinitrophenol in acute experiments did not result in any significant change in the succinoxidase activity of the livers of adult male albino rats.

Thyroxine, thyroglobulin and 2:4-dinitrophenol had no immediate significant influence *in vitro* on the succinoxidase activity of freshly prepared and dialyzed liver homogenates, and were also without influence on a purified preparation of succinic dehydrogenase.

It was observed that succinate affords considerable protection against the loss of succinoxidase activity which occurs on storage of liver homogenates at 4° C. The observation of Aloisi & Cavallini (1941) that thyroxine can activate *in vitro* preparations of succinoxidase which have lost activity as a result of cold storage could not be confirmed using liver homogenates.

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The Isolation and Partial Purification of 'Aerosporin'. By J. R. CATCH and R. FRIEDMANN. (*Wellcome Chemical and Wellcome Physiological Research Laboratories, Langley Court, Beckenham, Kent*)

Crude 'Aerosporin', prepared according to Ainsworth, Brown & Brownlee (1947), may be purified by fractional precipitation as the helianthate, successive portions of methyl orange solution being added to the neutral solution of the antibiotic in water or aqueous methanol. Helianthic acid in dilute aqueous pyridine is a more convenient reagent. The aerosporin is generally (although not invariably) precipitated first, followed by impurities which appear to be, like the antibiotic itself, basic peptides. The helianthate is an amorphous brown powder, almost completely insoluble in water and most solvents. The hydrochloride or sulphate may be recovered by treatment of a methanolic suspension with the appropriate mineral acid, removal of the sparingly soluble helianthic acid, and precipitation with anhydrous acetone. Further precipitation of the amorphous reineckate, and regeneration by treatment with Ag_2SO_4 and $BaCl_2$, yields a highly active hydrochloride, of which the homogeneity is still, however, uncertain. It is a colourless, amorphous deliquescent powder, m.p. 230–235° d. after softening, freely soluble in water and in methanol; it is laevorotatory, having $[\alpha]_{5461}^{20°}$ up to −42° in water. It is labile to alkali but fairly stable to acid. A positive biuret test is given and a positive ninhydrin reaction, both before and after acid hydrolysis. The Molisch, Sakaguchi, Pauly, and Hopkins-Cole reactions (for carbohydrates, monosubstituted guanidines, iminazoles, and indoles respectively) are negative. No characteristic absorption or fluorescence is shown in the ultra-violet. The elementary composition of the most active hydrochloride is C, 45·5; H, 7·7; N, 15·8; Cl, 14·0%. Sulphur and phosphorus are absent. The factor responsible for renal damage (Brownlee & Bushby, 1948) is generally still present, although some preparations contain relatively small amounts.

Many base precipitants and acid azo-dyes give precipitates with solutions of the antibiotic. The picrate and flavianate are amorphous and gummy. Treatment with benzoyl chloride or carbobenzoxychloride in aqueous sodium bicarbonate solution yields amorphous water-insoluble products, devoid of antibiotic activity.

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The Chemical Nature of 'Aerosporin'. Part II. Isolation of a New Natural Amino-Acid. By J. R. CATCH and TUDOR S. G. JONES. (*Wellcome Chemical Research Laboratories, Langley Court, Beckenham, Kent*)

The products of the acid hydrolysis of 'Aerosporin' have been shown in Part I (Jones, 1948) to contain a basic substance, reacting with ninhydrin in solution, but not identified with any of a large variety of amino-acids. This substance is readily isolated from the hydrolysate of the most active preparations of the antibiotic as the dipicrate, m.p. 184–185°. The yield is good but the removal of the basic constituent is not complete, as is shown by chromatography on paper. Micro-analysis indicates the empirical formula $C_{16}H_{16}N_8O_{16}$. Regeneration of the picrate with HCl, and repeated recrystallization from aqueous ethanol, gives a monohydrochloride as laths or plates, m.p. 228–230° d. It is optically inactive; elementary analysis is in agreement with the formula $C_4H_{11}N_2O_2Cl$. Recrystallization in the presence of excess HCl gives a salt, m.p. 195–198° d., which approaches the dihydrochloride in composition but is less well-defined than the monohydrochloride, to which it again reverts on recrystallization in the absence of excess HCl.

A sample of synthetic DL-$\alpha\gamma$-diaminobutyric acid, prepared from DL-glutamic acid by the method of Adamson (1939), gave corresponding derivatives having the same appearances, melting points, mixed melting points and elementary compositions as those obtained from the antibiotic. Chromatographic analysis on paper of the natural and synthetic amino-acids gave, alone or in admixture, spots indistinguishable in position and colour, using several solvents. The electrometric titration curves of the two were superimposable, and indicated that the pK_A values for the three functional groups are of the same order as those of lysine and ornithine.

The most active fractions of the antibiotic have already been shown to contain leucine and threonine as components (Jones, 1948); the third, hitherto unidentified component has now been characterized as DL-$\alpha\gamma$-diaminobutyric acid. It is believed that this is the first report of the occurrence of this acid in Nature.

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The Effect of Diabetic Blood Plasma on Hexokinase *in vitro*. By R. H. SMITH. (*Department Biochemistry, University College, London, W.C.* 1.) (Introduced by F. G. YOUNG)

De Wesselow & Griffiths (1936) observed that the blood plasma of elderly, obese, glycosuric patients, when injected into rabbits, diminished the hypoglycaemic action of insulin in a manner closely resembling that observed by other workers (e.g. Young, 1936) with anterior pituitary extracts. Similar instances of insulin antagonism by sera from insulin resistant patients have been reported by Glen & Eaton (1938), Felder (1946) and Lowell (1947).

In the light of the discovery of C. F. Cori and his co-workers (Colowick, Cori & Slein, 1947) concerning the ability of insulin to counteract the inhibition of hexokinase by anterior pituitary extract *in vitro*, it seemed possible that the anti-insulin effects observed by de Wesselow & Griffiths might be associated with the presence of a hexokinase inhibitor in the plasma.

Fractions of fresh blood plasma of a number of elderly, obese, glycosuric patients have been prepared by the alumina adsorption method and tested for hexokinase inhibiting properties following Colowick *et al.* (1947). In no instance has any significant insulin-reversible inhibition of brain hexokinase activity been obtained with these preparations.

The assistance of Prof. H. P. Himsworth and Dr M. E. Morgans in selecting the patients and obtaining the blood samples used in this work is gratefully acknowledged.

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Antagonism by Amino-acids of Renal Tubule Damaging Substances present in 'Aerosporin' Preparations. By G. BROWNLEE and EILEEN I. SHORT. (*Wellcome Physiological Research Laboratories, Langley Court, Beckenham, Kent*)

'Aerosporin' preparations may be more or less contaminated by a nephrotoxic impurity (Brownlee & Bushby, 1948). The lesions studied histologically in the rat and guinea pig appear to be restricted to the convoluted tubules. Estimates of the resultant proteinuria, in rats and dogs, have enabled semi-quantitative assays to be made, and potential antagonists to be tested. It is known that the nephrotoxic action of DL-serine is due to the unnatural isomer, D-serine (Artom, Fishman & Morehead, 1945), and the site of damage is the distal portion of the convoluted tubules (Wachstein, 1947*a*). Further, Wachstein (1947*b*) found DL-methionine and glutathionine, but not cysteine, thioglycollic acid or 2:3-dithiopropanol to protect against D-serine; DL-alanine, glycine, DL-threonine, and glycollic, butyric and pyruvic acids gave some protection.

We have confirmed the effects of these substances in rats and dogs by estimating the degree of proteinuria, and have further found that substances which antagonize the nephrotoxic action of D-serine, also antagonize the nephrotoxic activity of the impurity in the new antibiotic. Serine is absent from the toxic preparations, and the individual amino-acid components of the antibiotic (Catch & Jones, 1948) leucine, threonine and DL-$\alpha\gamma$-diaminobutyric acid are not themselves nephrotoxic.

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Fractionation of Diabetogenic Pituitary Extracts. By E. REID (Carnegie Research Fellow) and F. G. YOUNG. (*Department of Biochemistry, University College, London*)

Young (1939) described the fractionation of diabetogenic anterior pituitary extracts (ox) with ammonium sulphate. The diabetogenic and growth-promoting activities were found to associate in the 'pseudoglobulin' fraction, although in poor yield relative to crude extract. Alternative fractionation procedures are now being investigated, with the intact adult cat for the testing of diabetogenic activity (cf. Young, 1945). No appreciable diabetogenic activity is lost from alkaline saline extracts on dialysis, especially if the pH is maintained at approximately 8 during the dialysis. Good activity is present in the precipitate obtained by adjustment of the pH of the extract, thoroughly dialyzed at pH 8, to 6·0. This 'pH 6 precipitate' is quite heterogeneous, since some protein (the amount varying from one extract to another) precipitates at a higher pH and does not redissolve appreciably at pH 6·0. Diabetogenic activity is, in fact, associated with the protein which precipitates above pH 6·5, or possibly even higher (pH 6·75).

Published data for growth and lactogenic hormones suggests that these proteins tend to be soluble and insoluble, respectively, in the neighbourhood of pH 5·5, in the presence of a trace of inorganic salt. When solutions in 0·9 % NaCl of diabetogenic precipitates obtained from dialyzed extracts are acidified to pH 6·0, the activity remains in the supernatant fluid. Approximately 20–50 % of the activity of a crude extract can be recovered in such a 'saline supernatant' fraction, with a purification of approximately five-fold in terms of protein. If the pH is adjusted to 5·0 instead of 6·0, activity is divided between the precipitate and supernatant fluid.

The hexokinase-inhibiting pituitary factor is known to be adsorbed on to alumina and eluted by Na_2HPO_4 solution (Colowick, Cori & Slein, 1947). The diabetogenic factor behaves similarly, but adsorption on alumina is probably too non-specific a method to be of value in the purification of this factor.

In accordance with previous findings the diabetogenic and growth-promoting activities tend to associate; but it is likely that some factor more labile than growth hormone is implicated in the diabetogenic action of pituitary preparations on the *intact* adult dog or cat.

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The **266th Meeting** *of the Society was held in University College, Dublin, on Friday, 9 April* 1948, *at* 1.30 *p.m., when the following papers were read or taken as read:*

COMMUNICATIONS

Some Characteristics of the Nuclear Enzymes of Wheat. By R. GORDON BOOTH. (*Cereals Research Station, St Albans*)

The work of Peters (1945) on living animal tissues, and of Hutchinson, Greer & Thomas (1946) on living cereal grains indicated a close parallelism between the critical temperatures of damage (evidenced by lowered viability or death) in the two basic forms of living matter. Peters concludes that death is due either to irreversible inactivation of a nuclear enzyme system or to conversion of a cell membrane from its 'selective' living state to a non-selective semi-permeable type of membrane. This suggested that in the event of the first hypothesis being true, the most heat-labile enzyme systems of wheat might be found in or in close association with its cell nuclei and might be specifically concerned with the metabolism of nuclear contents.

Nucleotidases in wheat were estimated using an adenylic acid buffered substrate, the wheat extracts previously having been heat treated in the manner described by Booth (1944). Estimation of inorganic P was the measure of activity. The critical inactivation temperature of wheat nucleotidase was shown to approximate to 52·5°, this enzyme thus clearly

being no more heat labile than phosphomonoesterase (cf. Booth, 1944). An attempt was made to estimate the nucleosidase activity of wheat using both adenosine and guanosine as substrates, activity being measured by assay of total reducing sugar and of free pentose. Under the conditions employed, no nucleosidase activity could be detected even in the presence of activators such as KCN and cysteine.

Polynucleotidase proved to be the most heat labile of the three enzyme systems and was measured, using both yeast and thymus nucleic acids as substrate. The former substrate was most readily attacked, and the extent of depolymerization was measured by the use of the MacFadyen (1934) reagent.

The optimal pH for this enzyme in wheat proved to be 6·0 and its critical inactivation temperature was 48°.

This low critical inactivation temperature, however, is not sufficiently low to explain lowered viability and death phenomena in heated wheat.

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The Carcinogenic Activity of *N*-Ethyl-3:4:5:6-Dibenzcarbazole. By A. H. M. Kirby. (*Research Department, Glasgow Royal Cancer Hospital*)

3:4:5:6-Dibenzcarbazole (DBC) is a very active carcinogen for mouse skin; it is very active indeed for the subcutaneous tissues and also causes hepatoma. The effect of *N*-methylation has already been reported from this Department (Kirby & Peacock, 1946); a marked reduction of activity occurred, especially in regard to the subcutaneous tissues and the liver. Lacassagne, Buu-Hoi, Royer & Zajdela (1947) have shown *N*-methyl-1:2-benzcarbazole to be no less carcinogenic for mouse skin than is 1:2-benzcarbazole itself.

N-Ethyl-3:4:5:6-dibenzcarbazole (EtDBC) has been synthesized by the general method for carbazoles (Stevens & Tucker, 1923); it forms colourless needles from ethanol, m.p. 98·5°. (Found: C, 89·6; H, 5·74; N, 4·75%. Calc. for $C_{22}H_{17}N$: C, 89·5; H, 5·75; N, 4·74%.) A 0·2% solution in acetone was painted thrice weekly on the nape of the neck, after clipping away hair, in male and female mice of the Cba strain; two subcutaneous injections, one in the right flank and the second in the left 144 days later, were made with 0·3 mg. in 0·25 ml. tricaprylin and 0·5 mg. in 0·25 ml. arachis oil respectively. Comparative tests were made in parallel in mice of the same strain using DBC from the batch used in our previous work.

The results of painting EtDBC show that papillomas and squamous carcinomas developed in 8/9 mice painted for more than 200 days, compared with an average latent period of 110 days in the DBC group. One female mouse developed an angiosarcoma 218 days after the first injection of EtDBC; all the other mice (7 males, 5 females) survived longer than this (2 males and 1 female are still alive—400 days after first injection), but no tumours at the sites of injection have been found. DBC caused sarcomas in 8/10 mice surviving 110 days after the first injection. Mice painted with EtDBC had no hyperplastic liver changes; the DBC group had hepatoma in 1/12 mice. 1/13 mice injected subcutaneously with EtDBC has developed hepatoma; DBC-injected mice had an incidence of 20%.

It may be concluded that EtDBC is a much weaker carcinogen than is DBC when painted on the skin of mice, but very much weaker than DBC when injected subcutaneously; the action of EtDBC on the liver is only slight. As stock mice were used for tests with *N*-methyl-dibenzcarbazole, strict comparison is difficult, but it seems fair to say that EtDBC has a carcinogenic power of the same order as that of the *N*-methyl homologue.

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The Metabolism of Stilboestrol and Hexoestrol in Man. By F. H. MALPRESS. (*Department of Biochemistry, Queen's University, Belfast*)

Detailed quantitative studies on the excretion of synthetic oestrogens given in therapeutic doses to humans have not yet been reported, although Warren, Goulden & Robinson (1948), from observations based on the Dingemanse reaction, have concluded that the excretion of free stilboestrol in patients receiving a daily oral dose of 15 mg. could not exceed 5% of the dose given. Wilder Smith (1947), from indirect evidence, has claimed that less than 1% of the administered oestrogen is excreted in the free form, but as much as 50% as the monoglucuronide.

Determination of the urinary excretion of free and glucuronide forms after the injection or oral administration of stilboestrol and hexoestrol has now been made by a new and direct method (Malpress, 1948); preliminary results are given in Table 1.

Sulphate ester excretion is not accounted for in these results, but the evidence in the literature suggests that it is small, even in comparison with the glucuronide figure. In all cases excretion has been complete within 24 hr. of the last dose being given, and it seems probable that at these dosage levels more than 90% of the administered oestrogen undergoes radical metabolic change after intramuscular injection. The single result available after feeding stilboestrol suggests that, in its quantitative aspects at least, the metabolism of this oestrogen varies greatly with the route of its administration; further work is being done on this problem.

Table 1. *The excretion of stilboestrol and hexoestrol as free oestrogen (F) and glucuronide (G) in man, expressed as percentage total dose*

Patient	Oestrogen	5 mg. intramuscularly on 3 successive days		10 mg. intramuscularly single dose		5 mg. orally on 3 successive days	
		F	G	F	G	F	G
Mrs C.	Stilboestrol	1·3	3·2	0·6	4·0	—	—
,,	,,	1·0	8·1	—	—	—	—
,,	Hexoestrol	2·6	6·3	0·0	6·8	—	—
,,	,,	2·3	5·2	—	—	—	—
Mrs K.	Stilboestrol	3·9	5·0	—	—	—	—
Mrs T.	,,	—	—	—	—	71·4	14·4
Mr A.	,,	0·0*	7·7*	—	—	—	—

* Values slightly low owing to loss of one urine sample.

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The Solubility of Calcium Phosphate and Dental Tissues in Incubated Mixtures of Saliva and Flours of Different Extraction Rates. By G. N. JENKINS and M. G. FORSTER. (*Department of Physiology, Medical School, Newcastle-upon-Tyne*)

Osborn, Noriskin & Staz (1937*a*, *b*), impressed by the low incidence of dental caries among South African Bantus, postulated the existence of a protective factor against caries in unrefined cereals and sugars. These authors incubated teeth with saliva and either crude or refined carbohydrates, and by visual inspection found that the crude materials caused decalcification in a smaller proportion of the teeth than did the refined products.

In the present work tricalcium phosphate, ground enamel, ground dentine and whole non-carious human teeth have been incubated with saliva and wheat flours of 70, 85 and 100% extraction. The rates of solution of these materials were measured by estimating the calcium concentration in the incubated medium.

It was found that the amount of calcium dissolving from the powdered materials in the presence of wholemeal flour was invariably about 60–70% of the amount dissolving with flour of 70% extraction, thus supporting the suggestion of a protective factor in unrefined cereals. The calcium dissolved in the presence of 85% extraction flour was midway between that found with the other flours.

The results with whole teeth were less convincing. In one experiment of 3 months' duration, involving 32 teeth, the calcium estimations did suggest a reduced rate of decalcification with wholemeal and

85% extraction flour. Two other experiments of shorter duration showed virtually no differences. Macroscopic examination of the teeth in all three experiments failed to show any obvious difference in the rate at which decalcified patches formed and enlarged, contrary to the findings of Osborn *et al.*

The mechanism of the reduction in solubility caused by the higher extraction flours is not yet clear. It is not caused either by differences in pH produced from the various flours by the salivary bacteria, or by differences in the amount of calcium released from the flours.

Preliminary experiments with molasses and pure sucrose suggest that the former also contains a protective factor but the results indicate that its nature differs from that of the high extraction flours.

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Accumulation of L-Glutamic Acid in Isolated Brain Tissue. By J. R. STERN (National Research Council Fellow). (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

The method of Gale (1945) for the determination of L-glutamic acid has been applied to the study of the fate of L-glutamic acid added to brain slices. Under aerobic conditions $Q_{glutamate}$ (μl. L-glutamic acid disappearing from medium/mg. dry wt./hr.) was −2 to −2·5 for guinea-pig brain cortex slices. The addition of glucose to the medium increased $Q_{glutamate}$ by 50–120%. Oxaloacetate, fructose, pyruvate, phosphoglycerate plus adenosinetriphosphate (ATP) or phosphoglycerate (in order of decreasing efficacy) also increased $Q_{glutamate}$. The following substances had little or no effect; lactate, citrate, α-ketoglutarate, succinate, α-glycerophosphate, ATP and ammonia.

When L-glutamic acid was added alone, or with oxaloacetate, succinate or lactate, to brain slices, more L-glutamic acid than amino-N disappeared, presumably because of transamination reactions. In the presence of glucose, fructose, pyruvate or α-ketoglutarate, disappearance of L-glutamic acid was accompanied by an approximately equivalent loss of amino-N.

The concentration of L-glutamic acid within brain slices (initially 220–290 μl./g. wet wt.) decreased progressively on aerobic incubation without added substrates. This fall was largely or wholly prevented by glucose, lactate, pyruvate or oxaloacetate, but not by L-glutamic acid. The addition of both glucose and L-glutamic acid to the medium caused an accumulation of L-glutamic acid within the slices to levels of 500–600 μl./g. Thus, provided glucose was present, brain slices were able to concentrate L-glutamic acid to levels 2–2·5 times that of freshly excised tissue. The internal concentration of L-glutamic acid attained increased as its external concentration was increased, a 20-fold concentration being effected at low external concentration (16 μl./ml.) and a five- to sixfold concentration at higher external concentration (94 μl./ml.). Under the latter conditions a maximal internal concentration was reached in about 30 min. In these experiments glucose could be replaced wholly or partly by fructose, lactate or pyruvate, but not by α-glycerophosphate, phosphoglycerate, citrate, α-ketoglutarate, succinate, oxaloacetate, ATP, or phosphoglycerate plus ATP. Thus, the disappearance of L-glutamic acid from the medium was accounted for partly by accumulation within the slice and partly by chemical change.

The accumulation of L-glutamic acid was inhibited when the oxidation of glucose was inhibited by luminal, malonate, azide or 5×10^{-4}M iodoacetate. When lactate was oxidized, 5×10^{-4}M iodoacetate caused no inhibition. Anaerobically, no accumulation of L-glutamic acid was observed.

Under conditions employed in brain experiments L-glutamic acid did not accumulate in slices of guinea-pig kidney cortex or rat liver or in teased rat testis.

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The Purity of Adenosinetriphosphate Preparations by Enzymic Degradation. By K. BAILEY. (*Biochemical Laboratory, University of Cambridge*)

The wide use of adenosinetriphosphate (ATP) in enzyme reactions, and the advent of commercial preparations, have necessitated the development of purity tests by biological methods. Three enzyme systems are employed: (*a*) myosin-adenosinetriphosphatase (ATP-ase) (see Bailey, 1942); (*b*) ATP-ase plus the myokinase of Colowick & Kalckar (1943); (*c*) yeast inorganic pyrophosphatase (Bailey & Webb, 1944). With pure ATP, the free phosphate split by (*b*) is twice that by (*a*); if adenosinediphosphate (ADP) is present, the phosphate in (*b*) is greater than twice that in (*a*). Freshly prepared ATP contains no inorganic pyrophosphate, but on storage as the Ba salt over long periods (2 years), an almost complete breakdown, mostly to adenylic acid and pyrophosphate, and partly to ADP and free phosphate, has been observed. The nucleotide is best stored in solution as the Na salt (pH 6·8) at $-12°$.

The enzymic analysis of commercial and laboratory ATP specimens prepared from skeletal muscle (rabbit and ox) has given the following results: (1) Using the Lohmann-Needham method (D. M. Needham, 1942), ATP is obtained pure after four precipitations of the Ba salt; first as the dibarium salt, then as the monobarium salt from ethanol-HCl (Kerr, 1941), again as the di- and finally as the monobarium salt. (2) In N-HCl (1 ml. samples immersed in boiling water) the true acid-labile P is obtained only after 10 min. and not after the customary 7 min., although this latter period does suffice for inorganic pyrophosphate. As a percentage of the acid-labile P, the P liberated from the author's ATP by (*a*) is *c.* 46 %, by (*b*) 93–96 %, by (*c*) 0–2 % (in fresh preparations). The best commercial specimens give with (*a*) 40–42 %, (*b*) 80–85 %, (*c*) 0–10 %. (3) No ADP has been detected either in laboratory or commercial ATP preparations, nor in the insoluble Ba salts obtained from skeletal muscle in various stages of rigor. On the other hand, enzymic tests show the presence of considerable amounts of ADP in cardiac muscle (S. V Perry, personal communication), thus confirming the chemical characterization of both ATP and ADP in heart muscle by Lohmann & Schuster (1935). For all enzyme tests, the decomposition of the Ba salt of ATP on an exchange resin (see Polis & Meyerhof, 1946) is recommended for the removal not only of Ba, but also of metal impurities such as Fe^{+++}.

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The Concentration of Cozymase and Total Nicotinic Acid in Cat Oxyntic Cells. By NORAH M. BRADFORD, R. E. DAVIES, EDITH ELLIS and D. E. HUGHES. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

Maximally secreting mammalian oxyntic cells can have Q_{HCl} values greater than 1000μl./mg. dry wt./hr. and then secrete their own volume of acid juice every minute. If the secretory units in the pericanalicular zone of these cells (Crane, Davies & Longmuir, 1948) have turn-over numbers of 10^4 H^+ ions/secretory unit/min. (cf. McIlwain, 1946), there are 10^7 secretory units/oxyntic cell.

A hypothesis of HCl production (Crane *et al.*) requires that H atom transport by dehydrogenating enzymes is part of the mechanism by which H^+ ions are secreted, and hence that there are more than 10^7 such enzyme molecules/oxyntic cell. Experiments have therefore been made to determine the quantity of cozymase and of total nicotinic acid in cat oxyntic cells. Serial sections were cut with a freezing microtome (as described by Holter & Linderstrøm-Lang (1935) and Davenport (1939)) parallel to the surface of cylinders of cat gastric mucosa. The distribution of the various cells was determined by counts on sections regularly spaced through the thickness of the mucosa. The remaining sections were

Table 1. *Mean cozymase and total nicotinic acid concentrations in some cat cells and tissues*

Material	Mean conc. of cozymase (M)	Mean conc. of total nicotinic acid (M)
Oxyntic cells	3×10^{-3}	2×10^{-2}
Mucous cells	1×10^{-4}	2×10^{-4}
Muscularis mucosa	7×10^{-5}	3×10^{-4}
Other gastric cells	$1{\cdot}5 \times 10^{-4}$	5×10^{-4}
Liver	$6{\cdot}5 \times 10^{-4}$	$2{\cdot}4 \times 10^{-3}$
Thigh muscle	$3{\cdot}8 \times 10^{-4}$	$1{\cdot}1 \times 10^{-3}$
Ventricle	$2{\cdot}4 \times 10^{-4}$	$9{\cdot}6 \times 10^{-4}$
Kidney cortex	$1{\cdot}6 \times 10^{-4}$	$1{\cdot}3 \times 10^{-3}$
Spleen	$3{\cdot}8 \times 10^{-5}$	$5{\cdot}2 \times 10^{-4}$

used separately for microbiological assay; cozymase was estimated by the method of Axelrod & Elvehjem (1939) with the modifications of McIlwain & Hughes (1948) and total nicotinic acid by the method of Barton-Wright (1946). The amounts in the various cells were calculated from a comparison of the serial counts and assays.

The results show that cat oxyntic cells contain much more of these substances than the other cells of the gastric mucosa. Moreover, the content is higher than that of the other tissues examined by these techniques (see Table 1). This extremely high concentration of a co-dehydrogenase ($1{\cdot}5 \times 10^9$ molecules/cell) and its relation to the number of secretory units is in accordance with the high energy output of actively secreting oxyntic cells.

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The Chemical Nature of 'Aerosporin'. III. The Optical Configuration of the Leucine and Threonine Components. By TUDOR S. G. JONES. (*Wellcome Chemical Research Laboratories, Langley Court, Beckenham, Kent*)

Paper partition chromatograms of the products of the hydrolysis of 'Aerosporin' have been shown in Part I (Jones, 1948) to exhibit amino-acid spots in the positions corresponding to leucine and threonine. The method of Synge (1948) for the identification of D-amino-acids on paper has been applied to such chromatograms. The method consists of the application, by spraying, of a solution in 0·06 M-pyrophosphate buffer of the D-amino-acid oxidase of sheep's kidney (Negelein & Brömel, 1939), followed by incubation at 37° for 2½ hr. in a humid atmosphere of oxygen. The paper is then dried and developed with ninhydrin in the usual manner. By matching the quantity of amino-acid present on the paper with equal amounts of the pure D- and L-amino-acid and mixtures thereof, the removal of the D-amino-acid can be followed.

It was found that the component occupying the leucine position was completely removed by the oxidase indicating that this amino-acid has the D-configuration. On the other hand, the spot due to the threonine component remained substantially undiminished in intensity in agreement with a predominantly L-configuration. A small proportion of a control spot of D-threonine remained after treatment with the oxidase. No light was thrown on the configuration of the third component, $\alpha\gamma$-diaminobutyric acid, for the D-form in this case is unattacked by the oxidase under the conditions of the experiment (cf. Handler, Bernheim & Klein, 1941, for lysine).

The co-operation of Dr R. L. M. Synge is gratefully acknowledged.

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The Determination of Amino-acids by Polarographic Reduction of their Copper Complexes. By TUDOR S. G. JONES. (*Wellcome Chemical Research Laboratories, Langley Court, Beckenham, Kent*)

The copper phosphate reagent of Woiwod (1948) is suited to the polarographic determination of small amounts of α-amino-acids, and, in particular, those quantities which are found on paper following partition chromatography according to Consden, Gordon & Martin (1944).

For amino-acids in solution, a suitable small volume is added to the copper phosphate reagent diluted with an equal volume of 0·18 M-Na_2HPO_4 solution and, after removal of the dissolved oxygen by passage of inert gas, the polarogram is obtained. A polarogram of the diluted reagent is drawn on the same paper in the same manner. The increase in the limiting current for the region $-0{\cdot}7$ to $-1{\cdot}0$ V. is related to the amino-acid content of the solution polarographed. The relationship may be established

beforehand under standard conditions, or an *ad hoc* determination under the exact conditions of temperature, capillary constants, etc., obtaining in the cell, may be made by the addition of a comparable quantity of the amino-acid being determined, dissolved in a suitably small volume.

The current yield is mainly dependent upon the capillary constants. Amino-acids differ in the current given by equimolar solutions; this is a direct consequence of difference in the diffusion constants of the copper complexes. This is shown by the mean values for the current given below, obtained in a number of experiments, for 5 μM solutions in the diluted copper phosphate reagent of the following amino-acids: glycine, 0·136 μA.; leucine, 0·112 μA.; $\alpha\gamma$-diaminobutyric acid, 0·225 μA.; lysine, 0·09 μA. The current bears a linear relationship to concentration over a threefold range of concentration, e.g. for glycine over the concentration range 80–260 μmole/l., the current was 2·90 (S.D. ± 0·02) μA./1000 μmole/l. The reduction potentials of the copper complexes do not appear to differ significantly.

The bearing of these results on the determination of amino-acids on the paper chromatogram is discussed.

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The Fate of *N*-acyl Derivatives of Ambamide and V335. By R. L. Hartles and R. T. Williams.
(*Department of Biochemistry, University of Liverpool*)

Hartles & Williams (1947) indicated that the lack of systemic antibacterial activity shown by Ambamide (Marfanil) and V 335 was due to their rapid deamination and oxidation *in vivo* to inactive metabolites. It was also suggested that the antibacterial activity of the drugs might be preserved *in vivo* if the biologically sensitive —CH_2NH_2 group of these drugs could be protected by a group (such as an acyl group) which would be slowly removed *in vivo* releasing the active drug in the tissues. We have therefore synthesized a number of acylated derivatives of the type RCONHCH$_2$C$_6$H$_4$SO$_2$NH$_2$ (Ambamide derivatives) and RCONHCH$_2$C$_6$H$_4$SO$_2$CH$_3$ (V 335 derivatives) and studied their fate in the rabbit (RCO— being *formyl*, *acetyl*, *propionyl*, *butyryl*, *caproyl*, *caprylyl*, *capryl*, *lauryl*, *myristyl*, *palmityl* and *stearyl*).

For the V 335 derivatives the acetyl derivative was excreted unchanged in the urine; 75 % of the formyl and propionyl derivatives were excreted unchanged and 25 % deaminated. As the chain length of the acetyl group lengthened from C_4 to C_{10}, the amount of drug absorbed became progressively less, and at C_{12} and C_{14} none was absorbed and the drugs were found in the faeces. With the acyl derivatives C_4–C_{10} that part of the drug which was absorbed was completely deaminated and oxidized, no unchanged drug appearing in the urine. The compound which may be of therapeutic value is butyryl-V 335, for it appeared to be well absorbed, 95 % of the absorbed drug being excreted as *p*-methylsulphonyl-benzoic acid and only 5 % as unchanged material.

For the Ambamide derivatives, the formyl, acetyl and propionyl derivatives were excreted largely unchanged. The butyryl derivative was 33 % deaminated and oxidized and 66 % excreted unchanged; then the C_6, C_8 and C_{10} compounds became progressively less easily absorbed and finally the C_{12} and C_{14} compounds were not absorbed at all. The absorbed C_6, C_8 and C_{10} Ambamide derivatives were completely deaminated and oxidized to *p*-carboxybenzenesulphonamide.

It was to be expected that these acyl derivatives would have no antibacterial activity *in vitro*. Through Dr A. Spinks we were able to test a selection of these compounds *in vitro* against *C. welchii* and *M. tuberculosis*. Formyl-, butyryl-, caprylyl, myristyl-, and stearyl-Ambamide and formyl-, acetyl-, propionyl-, lauryl- and palmityl-V 335 were inactive against *C. welchii* and were not very encouraging against *M. tuberculosis*.

These compounds have not been tested *in vivo* under conditions where they might be expected to show activity.

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Carbonic Anhydrase as a Tool in Studying the Mechanism of Enzymic Reactions Involving H_2CO_3, CO_2 or HCO_3^-. By H. A. KREBS. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

Carbonic anhydrase was previously used as a tool by Ferguson & Roughton (1934) to demonstrate the presence of carbamino-haemoglobin and by Krebs & Roughton to show that CO_2, and not HCO_3^- or H_2CO_3, is the primary product of the action of urease on urea (Roughton, 1935). When carbonic anhydrase was added to the system yeast decarboxylase-pyruvate, the rate of CO_2 evolution was found to be retarded under certain conditions. This effect was studied with a manometric technique using Warburg manometers. To demonstrate an effect of carbonic anhydrase on carboxylase the enzyme must be highly active (decomposing >50 μl. pyruvate/ml./min. at 15°), pH must be near the neutral point, and a buffer, e.g. phosphate, must be present. No effects on carboxylase were found at pH 5·5. The observations are in agreement with the assumption that CO_2 is the primary form of carbonic acid arising during decarboxylation from pyruvate. Possible reasons for the discrepancy between these results and those of Conway & MacDonnell (1945) are discussed.

In work with carbonic anhydrase it is often of value to use specific inhibitors. Extending investigations of Mann & Keilin (1940) and of Davenport (1945) the inhibitory power of 25 sulphonamides was measured quantitatively. The concentrations of sulphonamides causing a 50% inhibition of carbonic anhydrase for some of the substances tested are shown in the following table, which will be discussed. The data refer to dilute solutions of carbonic anhydrase containing only negligible quantities of protein. The inhibitions were smaller in the presence of homogenized tissue.

Sulphonamide	Concentration causing 50% inhibition (10^{-7}M) 0°	15°
p-Toluenesulphonamide	1	2·4
Sulphanilamide	9	19
N^4-Acetylsulphanilamide	1·2	2·0
Marfanil	25	—
Thiophene-2-sulphonamide	3·0	3·8
2-Chloro-pyridine-5-sulphonamide	1·2	—
α-Naphthalene sulphonamide	1·6	2·0
Prontosil	0·06	—
Sulphamezathine	2200	4200
Sulphapyridine	510	1400
Sulphaguanidine	300	650

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A Periodic Classification in Spiral Form of the Biological Elements. By W. R. FEARON.

Tryptochrome: an Oxidation Derivative of Tryptophan. By W. R. FEARON.

The Urochrome Complex: Resolution of the Pigments Obtained by Oxidation in Presence of Zinc. By W. R. FEARON.

Concerning the Action of Yeast Carboxylase. By E. J. CONWAY and E. O'MALLEY. (*University College, Dublin*)

When yeast carboxylase acts on a high concentration of pyruvate in a space of constant volume and temperature, with adequate shaking, the following equation may be expected to apply over a short period, if direct formation of H_2CO_3 (or its ion) is occurring:

$$p=\frac{aRm}{\beta V_w}\left\{k't-\frac{k'}{\beta}(1-e^{-\beta t})\right\}. \qquad (1)$$

If CO_2 is directly formed, the equation may be written

$$p=\frac{aRm}{\beta V_w}\left\{k't+\frac{km}{\beta}(1-e^{-\beta t})\right\}. \qquad (2)$$

In these equations, $\beta=k'+km$; $k'=k_{H_2CO_3}$ ([H]/K') and $k=k_{CO_2}$ (K' being the first adjusted ionization constant of H_2CO_3); $m=V_w s/(V_w s+V_g)$; $s=[CO_2]_w/[CO_2]_g$; V_w and V_g = the volumes of the liquid and

gas phases; R = the rate of total CO_2 per minute; and a is a factor converting concentrations of gaseous CO_2 in the water phase to millimetres pressure reading p of the manometric fluid.

It follows that if carbonic anhydrase be present the ratio of the increase of pressure in its absence to the rate of pressure per minute when it is active is given by $-1/\beta$ for eq. (1), and by $+km/\beta k'$ for eq. (2), when t is relatively large (2 or 3 min. from pH 6·5 to 7·0 at 25°). Experiments at 25°, pH about 6·7, with laked blood (1 in 100) supplying carbonic anhydrase, with similar laked blood with cyanide—about N/200—in the companion flask inhibiting the carbonic anhydrase, showed an increased pressure with active carbonic anhydrase, to be expected from eq. (1). Allowance was made for the apparent cyanide effect as shown in control experiments without blood. (Some previous experiments at 37° and pH of 5·5 gave results quantitatively greater than could be expected from eq. (1).)

Further experiments carried out at 17° with phosphate buffer at a pH of 7·0, in which more diluted blood (1 in 500) was used to supply the carbonic anhydrase, and no blood or cyanide used in the companion flask, show the contrary result of a decreased pressure associated with carbonic anhydrase, and appear to agree with Prof. Krebs's findings (private communication).

The cause of the difference is being investigated.

Succinic Acid in Yeast. By T. Brady. (*University College, Dublin*)

When baker's yeast ferments glucose, in 5% unbuffered solution (1 : 0·6), it was shown that relatively large amounts of succinic acid enter the suspending fluid within 30–40 min. (Conway & O'Malley, 1943).

Further investigation has shown that the major portion of this acid is not formed directly from the glucose being fermented, but is already present in the cells. The succinic acid diffuses out from the cells so that the ratio of its concentrations within and without the cells is at or near to 0·1 (Conway & Brady, 1947).

Kleinzeller (1941) has shown that baker's yeast fermenting at a pH of 7·4 in bicarbonate medium can form succinic acid from glucose. Under the conditions used here, when the succinic acid which has passed into the suspending fluid is removed by centrifuging, the cells reform it to approximately the normal resting value.

Succinic acid or its precursor can be removed from the yeast cell by prolonged oxygenation. Short-period fermentation of yeast treated in this manner can give rise to acidity, with no release of succinic acid into the suspending fluid.

Experiments to show whether the succinic acid in the normal resting cell is present as the succinate ion or as some precursor, analytically determined as succinic acid, are in progress.

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Evidence for an Outer Metabolic Chamber in the Yeast Cell. By M. Downey and E. J. Conway. (*University College, Dublin*)

When yeast ferments glucose, succinic acid appears rapidly in the suspending fluid, but the ratio of succinic acid in the yeast cell to that outside is in the region 0·1–0·2. Similarly, when N/5-succinic acid is used as the suspending fluid (1 : 1) the acid enters rapidly to a similar ratio—0·17 in the average. So also does pyruvic acid which is steadily fermented at the same time. Over many hours succinic acid does not appear to penetrate to any greater extent than after a few minutes. A number of other acids, such as glyceric, malic, fumaric, etc., behave in a similar way to succinic and pyruvic acids, and their ratios when in N/5 strength outside range after an hour from 0·1 to 0·3 and are very little changed after several hours.

On the other hand, the group of ordinary aliphatic acids—acetic, propionic, butyric, etc.—appear to enter throughout the whole yeast cell, and so also does monoiodoacetic acid. With acetic acid the ratio, instead of being 0·18 as with succinic acid, is 1·7. As the external concentration is reduced the ratio increases, and the pH inside the resting cell can be calculated from the distribution, and is the same or close to that obtained by other methods.

If yeast is suspended (1 : 1) in solutions of varying strengths of glyceric acid, the ratio increases beyond 0·17 (but always only a small fraction for similar acetic acid levels), and assuming the initial volume of the outer chamber to correspond to 0·17 when the glyceric acid strength is high, the resting pH of the outer chamber can be roughly calculated. It would appear to be similar to that of the inner chamber, possibly somewhat lower. During fermentation there would appear to be a marked fall in the pH of this chamber as judged by the distribution ratio of small succinic acid concentrations.

Quantitative Aspects of the Storage of Vitamin A. By A. W. DAVIES and T. MOORE. (*Dunn Nutritional Laboratory, University of Cambridge*)

Observations on the percentage of vitamin A stored in the liver after large doses have given varying results. Thus Baumann, Rising & Steenbock (1934) found that only 10–20 % of the vitamin A which they had administered to rats was stored in the liver, while as much as 47 % was found by Golding, Kon & Campion (1936) to be stored in pigs. Recently, Lemley, Brown, Bird & Emmett (1947) have reported that over the range of dosage which they investigated the percentage of storage increased with the magnitude of the dose up to an optimum point, but declined when very heavy doses were given.

In the present work groups of rats were kept on a diet deficient in vitamin A until growth had ceased, and were then cured with small doses of carotene or vitamin A inadequate to allow storage. Graded single doses of vitamin A were then given as halibut-liver oil, and the animals were killed 48 hr. later. Vitamin A was estimated in the livers by the $SbCl_3$ method.

In one experiment doses of 100, 200, 400 and 600 i.u. were given, and the mean percentages stored were 0, 13, 15 and 38. Other experiments always showed the same tendency, although the results at each level of dosing were not always consistent. Thus in a second experiment the mean percentages of storage with doses of 200, 600 and 1000 i.u. were 2·4, 27 and 27. In a third experiment, in which the higher doses were divided over several days, the percentage storage after 1000, 2000, 3000 and 4000 i.u. were 25, 42, 50 and 50. By giving single doses of 60,000 i.u. the mean percentage of storage was raised to about 80, and about 70 % of the ingested vitamin remained in the livers of rats kept on the basal diet for 1–4 weeks after dosing.

Doses of 240,000 i.u. were usually not entirely consumed, but three rats ingested this amount over a period of 3 days, with fatal results. The percentages of storage in the animals were only 2·5–3·8. In a rat which was given 30,000 i.u. daily for 25 days only 10 % was stored.

Three factors appear to lower the percentage of storage of vitamin A: (1) At low doses about 100 i.u. is lost, or applied to other purposes, before storage in the liver commences. The proportion which is 'lost', although not necessarily the absolute amount 'lost', decreases as the dose is raised until a toxic level is reached. (2) With toxic overdoses a high proportion of the dose may disappear, and lower stores are found than would result from giving a dose just below the toxic level. (3) With prolonged massive dosing, without obvious toxic symptoms, the percentage of vitamin stored may be low even when a high concentration is attained in the liver. This is presumably due to 'saturation' of the liver with vitamin A.

It is difficult to place an upper limit on the concentration of vitamin A which may be attained in the rat's liver by heavy dosing, but values of more than 20,000 i.u. are at least uncommon. This level is 50–100 times greater than values common in wild rats, but concentrations of the same order have been found by Rodahl & Moore (1943) in polar bears and bearded seals, and are also common in whales and certain sharks. It is to be inferred that in the human subject and domestic animals actual saturation of the liver with vitamin A presumably never occurs under ordinary conditions of nutrition.

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Vitamin A in the Intestine (Pyloric Caeca) of the Halibut. By J. GLOVER and R. A. MORTON. (*Biochemistry Department, University of Liverpool*)

Many fish, e.g. the halibut, store large quantities of vitamin A. The intestine of the halibut, especially the pyloric caeca, is known to be very rich in the vitamin (Lovern & Morton, 1939). This is in sharp contrast to the intestines of mammals which contain little or no vitamin A. We have recently shown that carotene is transformed into vitamin A in the small intestine of the rat (Glover, Goodwin & Morton, 1947). Its detection there is difficult as it is rapidly removed after formation. In the halibut intestine, however, vitamin A accumulates; indeed, 50 % of the lipid extracts may be vitamin A esters. It was thought that an examination of fresh halibut intestine might provide a clue to the origin of this large concentration of vitamin A and also to its mode of absorption.

The pyloric caeca of a number of halibut were dissected into a series of layers (mucosa, tunica propria, stratum compactum and stratum granulosum, and muscle) immediately the fish were caught. Each

section of tissue was stored so as to preserve the vitamin A. The specimens were later examined for total, free and combined vitamin A. The results for the total vitamin indicated that the rich concentrate first appeared in the tunica propria. Over 40% of the lipid extracts from this layer was vitamin A esters. In the mucosa, 8–40% of the total vitamin present was in the alcohol form, whereas in the next layer, the tunica propria, the proportion of the free vitamin was more constant, 9–13%.

The calculated percentages of free fatty acids in equilibrium with the vitamin A ester and alcohol in each layer agreed moderately well with those experimentally determined on bulked samples of corresponding tissues (Lovern & Morton, 1939). This suggests that not only does each layer contain a lipase but that in all probability the same lipase is concerned in the formation of both glyceryl and vitamin A esters.

The origin of the high concentrates of vitamin A in the halibut may be explained in the following way: During the absorption of the fatty acids and vitamin A from the lumen of the pyloric caeca, a lipase must be continually effecting alternate synthesis and hydrolysis of the lipid esters as they pass through the mucosa and tunica propria. It is possible that the free fatty acids and vitamin A alcohol in the form of water-soluble complexes separately diffuse through the cell membranes. When glyceryl esters are formed in the tunica propria their further progress is checked by their slow absorption into the 'lymph system' which is much less well developed in fish than in mammals (van Herwerden, 1908; Greene, 1913). Fatty acids, however, diffuse into this layer from the mucosa at a much more rapid rate than the esters can be removed, and are then synthesized by lipase into more esters which accumulate. However, a certain amount of free fatty acid still remains in the diffusible form because of the lipase equilibrium established. Its concentration will rise as the quantity of glyceride increases until it reaches a saturation level when the entry of further amounts of fatty acid is prevented.

If free vitamin A, either ingested preformed or produced from carotenoids, enters the tunica propria it may be esterified by the lipase utilizing some fatty acid already present there. As more vitamin A presents itself, further ester can be formed until the level of vitamin A alcohol complex in the tunica propria maintained by the lipase also reaches a saturation level and prevents further entry of free vitamin A. Theoretically this stage is reached when vitamin A ester attains a concentration of *c.* 50% of the total lipids.

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Microdiffusion Methods. Improvements in the Determination of Carbon Monoxide and of Alcohol. By M. T. Ryan, J. Nolan and E. J. Conway.

Gray & Sandiford (1946) have used a microdiffusion procedure for determining carbon monoxide in blood, in which acid palladium chloride is reduced in the central chamber, an equivalent amount of free palladium being formed. The residual palladium after the use of aluminium sulphate to coagulate the colloidal metal, filtering, washing several times and making up to 50 ml. volume with some gum ghatti and potassium iodide, is determined spectrophotometrically. Instead of this spectrophotometric procedure, use is made of the fact that when the CO reduces the palladium salt, an equivalent amount of free HCl is formed, which can be directly titrated after removal of a portion into a small tube with a drop of bromophenol blue solution. Interference with the titration by colloidal palladium is prevented by incorporating some magnesium chloride in the absorbing fluid in the central chamber.

Many analyses of blood saturated with CO gave a standard deviation of 0·08 vol. %, and with different blood samples the results obtained (using coal gas as the source of CO) did not differ appreciably from the results obtained using the complete method of Horvath & Roughton (1942). The method should now be suitable for determining the oxygen combining capacity of blood as well as its CO content.

Alcohol. In the microdiffusion procedure of Winnick (1942) alcohol diffuses from 1·0 to 0·25 ml.

in the outer chamber into 0·5 ml. of acid dichromate solution in the central chamber, where it is oxidized, the residual dichromate being determined iodimetrically. By taking more advantage of the fact that the rate of absorption is inversely proportional to the volume of a given fraction of the alcohol introduced in the outer chamber, also that saturated potassium carbonate much increases the alcohol tension, practically full absorptions can be obtained in 1 hr. at room temperature.

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The Source of the Hydrogen Ions in the High Acid Formation by Yeast in Presence of KCl. By E. J. Conway and T. Brady.

It was shown (Conway & O'Malley, 1942; 1943; 1944; 1946) that a high acidity can be developed in the suspending fluid when baker's yeast ferments unbuffered glucose solution in presence of KCl, the acidity arising from an interchange of K and H ions.

It was found later (Conway & Brady, 1947) that succinic acid anions and those associated with acid-labile carbon dioxide could account quantitatively for the absorbed K ions, or H ions exchanged. The inference was drawn at first that acids yielding these anions were sources of the H ions exchanged. This conclusion, however, is open to certain qualifications, though strong evidence can be provided that under certain conditions succinic acid provides directly a fraction of the hydrogen ions exchanged.

Concerning the increase in acid-labile carbon dioxide, when K exchanges for H ions, this increase is quantitatively similar to the absorbed K ions when succinic acid is very depleted by prior oxygenation. Evidence for this fraction being bicarbonate appears in its barium insolubility in presence of dimethylamine. Even if decarboxylation produced carbonic acid directly serious difficulties would arise in the quantitative interpretation, both with respect to the source of the hydrogen ions that might arise in such decarboxylation, and to the rate at which carbonic acid would be broken down. The nature and action of the increased acid-labile carbon dioxide is open to further interpretation.

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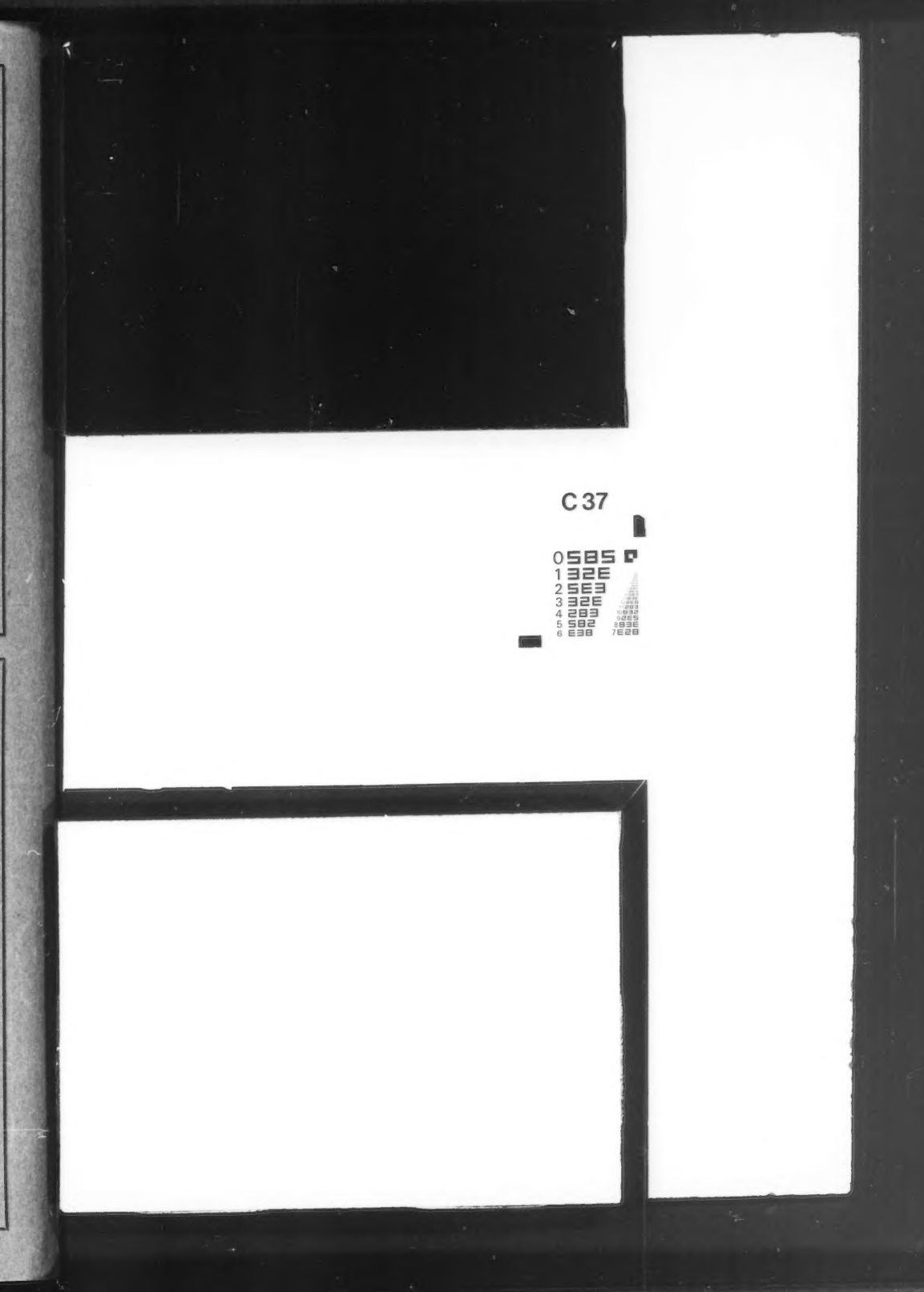
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